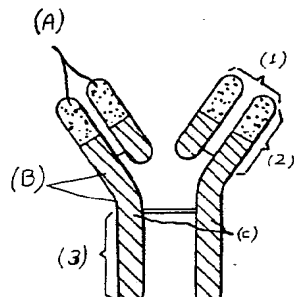# PCT

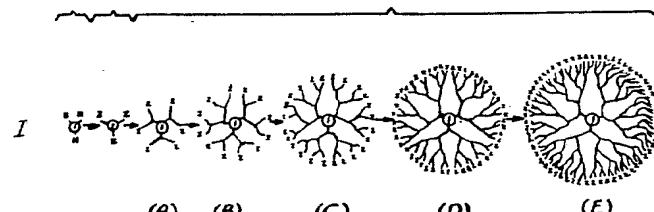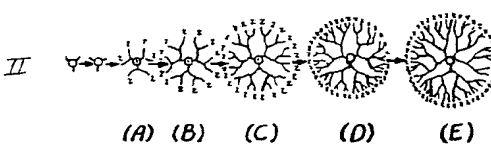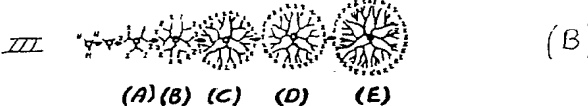## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(54) Title: STARBURST CONJUGATES

(57) Abstract

Starburst conjugates which are composed of at least one starburst polymer in association with at least one unit of a carried pharmaceutical material have been prepared. These conjugates have particularly advantageous properties due to the unique characteristics of the starburst polymer.

STARBURST CONJUGATES

        The present invention concerns the use of dense
star polymers as carriers for pharmaceutical materials.
In recent years polymers referred to as dense star
5   polymers or starburst polymers have been developed. It
has been found that the size, shape and properties of
these dense star polymers or starburst polymers can be
molecularly tailored to meet specialized end uses.
Starburst polymers have significant advantages which
10   can provide a means for the delivery of high concen-
trations of carried material per unit of polymer, con-
trolled delivery, targeted delivery and/or multiple
species delivery or use.

15   In its broadest aspect, the present invention
is directed to polymer conjugate materials comprising
dense star polymers or starburst polymers associated
with desired materials (hereinafter these polymer
conjugates will frequently be referred to as "starburst
20   conjugates" or "conjugates"), process for preparing
these conjugates, compositions containing the

25

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conjugates, and methods of using the conjugates and
compositions.

     The conjugates of the present invention are
5   suitable for use in a variety of applications where
specific delivery is desired, and are particularly
suited for the delivery of biologically active agents.
In a preferred embodiment of the present invention, the
starburst conjugates are comprised of one or more
10 starburst polymers associated with one or more
bioactive agents.

     The starburst conjugates offer significant
benefits over other carriers known in the art due to
15 the advantageous properties of the starburst polymers.
Starburst polymers exhibit molecular architecture
characterized by regular dendritic branching with
radial symmetry. These radially symmetrical molecules
are referred to as possessing "starburst topology".
20 These polymers are made in a manner which can provide
concentric dendritic tiers around an initiator core.
The starburst topology is achieved by the ordered
assembly of organic repeating units in concentric,
dendritic tiers around an initiator core; this is
25 accomplished by introducing multiplicity and self-
replication (within each tier) in a geometrically
progressive fashion through a number of molecular
generations. The resulting highly functionalized
30 molecules have been termed "dendrimers" in deference to
their branched (tree-like) structure as well as their
oligomeric nature. Thus, the terms starburst oligomer
and starburst dendrimer are encompassed within the term
starburst polymer. Topological polymers, with size and
35 shape controlled domains, are dendrimers that are
covalently bridged through their reactive terminal

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groups, which are referred to as starburst "bridged dendrimers." The term bridged dendrimer is also encompassed within the term "starburst polymer".

5   The following description of the figures aid in understanding the present invention.

Figure 1 depicts various generations of starburst dendrimers.

10   Figure 2A depicts a dendrimer having unsymmetrical (unequal) branch junctures.

Figure 2B depicts a dendrimer having symmetrical (equal) branch junctures.

15   Figure 3 depicts dendrimer sizes relative to antibody dimensions.

Figure 4 shows carbon-13 spin lattice relaxation times 20   (T_1) for aspirin incorporated into various dendrimer generations. (Example 1)

Figure 5 shows the results of the dynamic analysis of Example 2.

25   Figure 6 shows the influence of generation 6.5 dendrimer on the dialysis rate of pseudoephedrine at pH 9.5 from Example 2.

30   Figure 7 shows the effect of dendrimer hydrolysis on the permeability of pseudoephedrine of Example 3.

Example 8 Comparison of Percent Salicylic Acid released into the receptor compartment in the presence of 35   starburst polymer (Gen = 4.0) at pH 5.0 and 6.65 with salicyclic acid control, Example 4.

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Figure 9 Comparison of percent salicyclic acid lost
from donor compartment with starburst polymer (Gen =
4.0) in receptor compartment at pH 8.0 to salicyclic
acid content, Example 4.

5

Figure 10 Comparison of percent salicyclic acid lost
from donor compartment in presence of starburst polymer
(Gen =4.5) to salicyclic acid control, Example 4.

10   The starburst polymers are illustrated by
Figure 1 wherein (I) represents an initiator core (in
this figure a tri-functional initiator core shown by
the far left drawing,); Z represents a terminal group,
shown in the first instance by the second drawing from
15   the left, referred to as a starbranched oligomer; A, B,
C, D, and E represent particular molecular generations
of starburst dendrimers; and $(A)_n$, $(B)_n$, $(C)_n$, $(D)_n$,
and $(E)_n$ represent starburst bridged dendrimers.

20   The starburst dendrimers are unimolecular
assemblages that possess three distinguishing
architectural features, namely, (a) an initiator core,
(b) interior layers (generations, G) composed of
repeating units, radially attached to the initiator
25   core, and (c) an exterior surface of terminal
functionality (i.e., terminal functional groups)
attached to the outermost generation. The size and
shape of the starburst dendrimer molecule and the
functional groups present in the dendrimer molecule can
30   be controlled by the choice of the initiator core, the
number of generations (i.e., tiers) employed in
creating the dendrimer, and the choice of the repeating
units employed at each generation. Since the
35   dendrimers can be readily isolated at any particular

-5-

generation, a means is provided for obtaining
dendrimers having desired properties.

The choice of the starburst dendrimer
5   components affects the properties of the dendrimers.
The initiator core type can affect the dendrimer shape,
producing (depending on the choice of initiator core),
for example, spheroid-shaped dendrimers, cylindrical or
rod-shaped dendrimers, ellipsoid-shaped dendrimers, or
10 mushroom-shaped dendrimers. Sequential building of
generations (i.e., generation number and the size and
nature of the repeating units) determines the
dimensions of the dendrimers and the nature of their
interior.
15
Because starburst dendrimers are branched
polymers containing dendritic branches having
functional groups distributed on the periphery of the
branches, they can be prepared with a variety of
20 properties. For example, the macromolecules depicted
in Figure 2A, and the starburst dendrimers, such as
those depicted in Figure 2B can have distinct
properties due to the branch length. The dendrimer
type shown in Figure 2A possesses unsymmetrical
25 (unequal segment) branch junctures, exterior (i.e.,
surface) groups (represented by Z'), interior moieties
(represented by Z) but much less internal void space.
The preferred dendrimer type shown in Figure 2B
30 possesses symmetrical (equal segment) branch junctures
with surface groups (represented by Z'), two different
interior moieties (represented respectively by X and Z)
with interior void space which varies as a function of
the generation (G). The dendrimers such as those
35 depicted in Figure 2B can be advanced through enough
generations to totally enclose and contain void space,

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to give an entity with a predominantly hollow interior
and a highly congested surface. Also, starburst
dendrimers, when advanced through sufficient
generations exhibit "starburst dense packing" where the
5   surface of the dendrimer contains sufficient terminal
moieties such that the dendrimer surface becomes
congested and encloses void spaces within the interior
of the dendrimer. This congestion can provide a
molecular level barrier which can be used to control
10   diffusion of materials into or out of the interior of
the dendrimer.

Surface chemistry of the dendrimers can be
controlled in a predetermined fashion by selecting a
15   repeating unit which contains the desired chemical
functionality or by chemically modifying all or a
portion of the surface functionalities to create new
surface functionalities. These surfaces may either be
targeted toward specific sites or made to resist uptake
20   by particular organs or cells e.g. by reticuloendo-
thelial cells.

In an alternative use of the starburst
dendrimers, the dendrimers can themselves be linked
25   together to create polydendric moieties (starburst
"bridged dendrimers") which are also suitable as
carriers.

In addition, the dendrimers can be prepared so
30   as to have deviations from uniform branching in
particular generations, thus providing a means of
adding discontinuities (i.e., deviations from uniform
branching at particular locations within the dendrimer)
35   and different properties to the dendrimer.

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The starburst polymers employed in the
starburst conjugates of the present invention can be
prepared according to methods known in the art, for
example, U. S. Patent 4,587,329.

5   Dendrimers can be prepared having highly
uniform size and shape and most importantly allow for a
greater number of functional groups per unit of surface
area of the dendrimer, and can have a greater number of
10   functional groups per unit of molecular volume as
compared to other polymers which have the same
molecular weight, same core and monomeric components
and same number of core branches as the starburst
polymers. The increased functional group density of
15   the dense starburst polymers may allow a greater
quantity of material to be carried per dendrimer.
Since the number of functional groups on the dendrimers
can be controlled on the surface and within the
interior, it also provides a means for controlling the
20   amount of bioactive agent to be delivered per den-
drimer. In a particularly preferred embodiment of the
present invention the starburst polymers, particularly
the starburst dendrimers, are targeted carriers of
25   bioactive agents capable of delivering the bioactive
agents to a particular target organism or to a
particular determinant or locus in a target organism.

An analogy can be made between early generation
30   starburst dendrimers (i.e. generation = 1-7) and
classical spherical micelles. The dendrimer-micelles
analogy was derived by comparing features which they
had in common such as shape, size and surface.

35

Table I

Parameter	Regular Classical Micelles	Starburst Dendrimers
Shape	Spherical	Spherical
Size (diameter)	20-60Å	17-67Å
Surface aggregation number	4-202	Z=6-192 (Z is the number of surface groups)(generation = 2-7)
area/surface group (Å^2)	130-80Å^2	127-75Å^2

(1Å = 10^{-1} nm; 1Å^2 = 10^{-2} nm^2)

In Table I, the shape was verified by scanning
transmission electron micrographs (STEM) microscopy and
intrinsic viscosity (η) measurements. The size was
verified by intrinsic viscosity [η] and size exclusion
chromatography (SEC) measurements. The surface
aggregation numbers were verified by titrmetry and high
field NMR. The area/surface group was calculated from
SEC hydrodynamic measurements.

The first five generations of starburst
polyamidoamine (PAMAM) dendrimers are microdomains
which very closely mimic classical spherical micelles
in nearly every respect (i.e. shape, size, number of
surface groups, and area/surface groups). A major
difference, however, is that they are covalently fixed
and robust compared to the dynamic equilibrating nature
of micelles. This difference is a significant
advantage when using these microdomains as
encapsulation devices.

As further concentric generations are added beyond five, congestion of the surface occurs. This congestion can lead to increased barrier characteristics at the surface and manifests itself as a smaller surface area per head (surface) group as shown in Table II.

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Table II

PAMAM Dendrimer Features vs. Generation

Generations	1	2	3	4	5	6	7	8	9
# of surface groups, Z	3	6	12	24	48	96	192	384	768
Molecular wt.	275	875	2411	5147	10,619	21,563	43,541	87,227	174,779
Diameter* measured SEC	10.4Å	15.8Å	22Å	31Å	40Å	53Å	67Å	76Å	88Å
Surface area per dendrimer	$366Å^2$	$783Å^2$	$1519Å^2$	$3018Å^2$	$5024Å^2$	$8,820Å^2$	$14,096Å^2$	$18,136Å^2$	$36,083Å^2$
Surface area per Z group	$122Å^2$	$131Å^2$	$127Å^2$	$126Å^2$	$104Å^2$	$92Å^2$	$73Å^2$	$47Å^2$	$32Å^2$
Distance between Z groups	12.4Å	12.8Å	12.7Å	12.6Å	11.5Å	10.8Å	9.8Å	7.75Å	6.28Å
Void Volume	$311.6Å^3$	$1,470.2Å^3$	$4,737.9Å^3$	$11,427.0Å^3$	---	---	---	---	---

* Hydrodynamic diameters determined by size exclusion chromatogaphy measurements calibrated against monodisperse ($\frac{Mw}{Mn}$ = 1.02) polyethyleneoxide standards.

$1Å = 10^{-1}$ nm; $1Å^2 = 10^{-2}$ nm^2; $1Å^3 = 10^{-3}$ nm^3.

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For example, amine terminated generations 5.0,
6.0, 7.0, 8.0 and 9.0 have decreased surface areas of
104, 92, 73, 47 and 32$\mathring{A}^2$ per Z group, respectively.
This characteristic corresponds to a transition from a
less congested micelle-like surface to a more congested
bi-layer/monolayer barrier-like surface normally
associated with vesicles (liposomes) or Langmuir-
Blodgett type membranes.

If this surface congestion is occurring, the
change in physical characteristics and morphology
should be observed as the generations increase from the
intermediate generation (6-8) to the more advanced
generations (9 or 10). The scanning transmission
electron micrographs (STEM) for generations = 7.0, 8.0
and 9.0 were obtained after removing the methanol
solvent from each of the samples to provide colorless,
light yellow solid films and staining with osmium
tetraoxide. The morphological change predicted
occurred at the generation G=9.0 stage. The
microdomains at generation = 9.0 measure about 33$\mathring{A}$ in
diameter and are surrounded by a colorless rim which is
about 25$\mathring{A}$ thick. Apparently methanolic solvent has
been entrapped within the 25$\mathring{A}$ outer membrane-like
barrier to provide the dark stained interior. Thus, at
generation = 9.0, the starburst PAMAM is behaving
topologically like a vesicle (liposome). However, this
starburst is an order of magnitude smaller and very
monodispersed compared to a liposome. Consequently,
the present dendrimers can be used to molecularly
encapsulate solvent filled void spaces of as much
diameter as about 33$\mathring{A}$ (volume about 18,000$\mathring{A}^3$) or more.
These micelle sized prototypes appear to behave like a
covalently fixed liposome in this advanced generation

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stage.   This behavior enables these prototypes to serve
as drug delivery agents or as carriers for non-
chelating radionuclides in starburst antibody
conjugates for the treatment of various mammalian
5   diseases.

Dendrimers suitable for use in the conjugates
of the present invention include the dense star
polymers or starburst polymers described in U. S.
10   Patents 4,507,466, 4,558,120, 4,568,737 and 4,587,329.

In particular, the present invention concerns a
starburst conjugate which comprises at least one
starburst polymer associated with at least one carried
15   pharmaceutical material. Starburst conjugates included
within the scope of the present invention include those
represented by the formula:
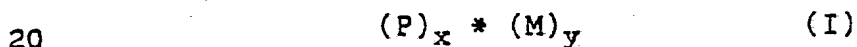
20                      $(P)_x * (M)_y$ (I)

wherein each P represents a dendrimer;

x represents an integer of 1 or greater;

25   each M represents a unit (for example, a molecule,
atom, ion, and/or other basic unit) of a carried
pharmaceutical material, said carried pharmaceutical
material can be the same carried pharmaceutical
material or a different carried pharmaceutical
30   material, preferably the carried pharmaceutical
material is a bioactive agent;

y represents an integer of 1 or greater; and

35

* indicates that the carried pharmaceutical material is associated with the dendrimer.

Preferred starburst conjugates of formula (I) are those in which M is a drug, radionuclide, chelant, chelated metal, toxin, antibody, antibody fragment, antigen, signal generator, signal reflector, or signal absorber; particularly preferred are those in which $x=1$, and $y=2$ or more.

Also included are starburst conjugates of formula (I) wherein the starburst dendrimers are covalently linked together, starburst bridged dendrimers, optionally via linking groups, so as to form polydendric assemblages (i.e., where $x>1$). Uses of these starburst bridged dendrimers include topical controlled release agents, radiation synovectomy, and others.

As used herein, "associated with" means that the carried material(s) can be encapsulated or entrapped within the core of the dendrimer, dispersed partially or fully throughout the dendrimer, or attached or linked to the dendrimer, or any combination thereof. The association of the carried material(s) and the dendrimer(s) may optionally employ connectors and/or spacers to facilitate the preparation or use of the starburst conjugates. Suitable connecting groups are groups which link a targeting director (i.e., T) to the dendrimer (i.e., P) without significantly impairing the effectiveness of the director or the effectiveness of any other carried material(s) (i.e., M) present in the starburst conjugate. These connecting groups may be cleavable or non-cleavable and are typically used in order to avoid steric hindrance between the target

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director and the dendrimer, preferably the connecting
groups are stable (i.e., non-cleavable). Since the
size, shape and functional group density of the
starburst dendrimers can be rigorously controlled,
5   there are many ways in which the carried material can
be associated with the dendrimer. For example, (a)
there can be covalent, coulombic, hydrophobic, or
chelation type association between the carried
material(s) and entities, typically functional groups,
10 located at or near the surface of the dendrimer; (b)
there can be covalent, coulombic, hydrophobic, or
chelation type association between the carried
material(s) and moieties located within the interior of
the dendrimer; (c) the dendrimer can be prepared to
15 have an interior which is predominantly hollow allowing
for physical entrapment of the carried materials within
the interior (void volume), wherein the release of the
carried material can optionally be controlled by
20 congesting the surface of the dendrimer with diffusion
controlling moieties; or (d) various combinations of
the aforementioned phenomena can be employed.

      Dendrimers, herein represented by "P", include
25 the dense star polymers described in U. S. Patent
4,507,466, 4,558,120, 4,568,737 or 4,587,329.

      Carried pharmaceutical materials, herein
represented by "M", which are suitable for use in the
30 starburst conjugates include any materials in vivo or
in vitro use for diagnostic or therapeutic treatment
which can be associated with the dense star dendrimer
without appreciably disturbing the physical integrity
of the dendrimer, for example, drugs such as
35 antibiotics, analgesics, hypertensives, cardiotonics,
and the like such as acetaminaphen, acyclovir, alkeran,

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amikacin, ampicillin, aspirin, bisantrene, bleomycin,
neocardiostatin, chloroambucil, chloramphenicol,
cytarabine, daunomycin, doxorubicin, fluorouracil,
gentamycin, ibuprofen, kanamycin, meprobamate,
methotrexate, novantrone, nystatin, oncovin,
phenobarbital, polymyxin, probucol, procarbabizine,
rifampin, streptomycin, spectinomycin, symmetrel,
thioguanine, tobramycin, trimethoprim, valban; and
toxins such as diphtheria toxin, gelonin, exotoxin A,
abrin, modeccin, ricin, or toxic fragments thereof;
metal ions such as the alkali and alkaline-earth
metals; radionuclides such as those generated from
actinides or lanthanides or other similar transition
elements or from other elements, such as ^{67}Cu, ^{90}Y,
^{111}In, ^{131}I, ^{186}Re, ^{105}Rh, ^{99m}Te, ^{67}Ga, ^{153}Sm, ^{159}Gd,
^{175}Yb, ^{177}Lu, ^{88}Y, ^{166}Ho, ^{115m}In, ^{109}Pd, ^{82}Rb, ^{194}Ir,
^{140}Ba, ^{149}Pm, ^{199}Au, ^{140}La, and ^{188}Re; signal
generators such as fluorescing entities; signal
reflectors such as paramagnetic entities for example
^{56}Fe, Gd, ^{55}Mn; chelated metal such as any of the
metals given, whether or not they are radioactive when
associated with a chelant; signal absorbers such as
electron beam opacifiers; antibodies including
monoclonal antibodies and anti-idiotype antibodies;
antibody fragments; hormones; biological response
modifiers such as interleukins, interferons, viruses
and viral fragments; diagnostic opacifiers; and
fluorescent moieties. Carried pharmaceutical materials
include scavenging agents such as chelants, antigens,
antibodies or any moieties capable of selectively
scavenging therapeutic or diagnostic agents.

Preferably the carried pharmaceutical materials
are bioactive agents. As used herein, "bioactive"

refers to an active entity such as a molecule, atom,
ion and/or other entity which is capable of detecting,
identifying, inhibiting, treating, catalyzing,
controlling, killing, enhancing or modifying a targeted
5   entity such as a protein, glycoprotein, lipoprotein,
lipid, a targeted cell, a targeted organ, a targeted
organism [for example, a microorganism or animal
(including mammals such as humans)] or other targeted
moiety.
10

     The starburst conjugates of formula (I) are
prepared by reacting P with M, usually in a suitable
solvent, at a temperature which facilitates the
association of the carried material (M) with the
15   starburst dendrimer (P).

     Suitable solvents are solvents in which P and M
are at least partially miscible and inert to the
formation of the conjugate. If P and M are at least
20   partially miscible with each other, no solvent may be
required. When desired, mixtures of suitable solvents
can be utilized. Examples of such suitable solvents
are water, methanol, ethanol, chloroform, acetonitrile,
25   toluene, dimethylsulfoxide and dimethylformamide.

     The reaction conditions for the formation of
the starburst conjugate of formula (I) depends upon the
particular dendrimer (P), the carried pharmaceutical
30   material (M), and the nature of the bond (*) formed.
For example if P is the PEI (polyethyleneimine)
starburst dendrimer with a methylene carboxylate
surface, M is a radionuclide, e.g. yttrium, then the
reaction is conducted at room temperature in water.
35   However, if P is an ester terminated PAMAM starburst
dendrimer, M is aspirin, then the reaction is conducted

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at room temperature in chloroform. Typically, the
temperature can range from room temperature to reflux.
The selection of the particular solvent and temperature
will be apparent to one skilled in the art.

5   The ratio of M:P will depend on the size of the
dendrimer and the amount of carried material. For
example, the molar ratio (ratio of moles) of any ionic
M to P usually is 0.1-1,000:1, preferably 1-50:1, and
10   more preferably 2-6:1. The weight ratio of any drug or
toxin M to P usually is 0.1-5:1, and preferably 0.5-
3:1.

       When M is a radionuclide, there are three ways
15   the starburst conjugate can be prepared, namely: (1) P
can be used as a chelant. For example a
methylenecarboxylate surface PEI or PAMAM will chelate
a metal such as yttrium or indium. (2) A chelate can
be covalently bonded to P. For example, an amine
20   terminated PEI starburst dendrimer can be reacted with
1-(p-isothiocyanatobenzyl)diethylenetriaminepenta-
acetic acid and then chelated, or a complex such as
rhodium chloride chelated with isothiocyanatobenzyl-
2,3,2-tet can be reacted. (3) A prechelated
25   radionuclide can be associated with P by hydrophobic or
ionic interaction.

       Particularly preferred starburst conjugates are
those conjugates which contain a target director
30   (herein designated as "T") and which are represented by
the formula:

$$(T)_e \ * \ (P)_x \ * \ (M)_y \qquad\qquad (II)$$

35

wherein

each T represents a target director;

5   e represents an integer of 1 or greater; and

P, x, *, M, and y are as previously defined herein.

Preferred among the starburst conjugates of formula
10 (II) are those in which M is a drug, radionuclide,
chelator, chelated metal, toxin, signal generator,
signal reflector, or signal absorber. Also preferred
conjugates are those conjugates in which e=1 or 2; and
those in which x=1 and y=2 or more. Particularly
15 preferred conjugates are those in which x=1, e=2, y=2
or more and M and T are associated with the polymer via
the same or different connectors.

The starburst conjugates of formula (II) are
20 prepared either by forming T*P and then adding M or by
forming P*M and then adding T. Either reaction scheme
is conducted at temperatures which are not detrimental
to the particular conjugate component and in the
presence of a suitable solvent when required. To
25 control pH, buffers or addition of suitable acid or
base is used. The reaction conditions are dependent on
the type of association formed(*), the starburst
dendrimer used (P), the carried pharmaceutical material
(M), and the target director (T). For example, when T
30 is a monoclonal antibody and M is a radionuclide, the
T*P association is done through a functional group such
as an isothiocyanate in water or in water with an
organic modifier such as acetonitrile or
35 dimethylformamide. Usually, the conjugation is done in
a buffer at pH 7-10, preferably pH 8.5-9.5. The formed

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conjugate is then chelated with a radionuclide such as
yttrium acetate, preferably at room temperature.
Alternatively, P and M can be chelated, usually in
water, before conjugation to T. The conjugation with T

5   is carried out in a suitable buffer.

The ratio of T:P is preferably 1:1, especially
when T is an antibody or fragment. The ratio of M:P
will be as before.

10

Target directors capable of targeting the
starburst conjugates are entities which when used in
the starburst conjugates of the present invention
result in at least a portion of the starburst

15   conjugates being delivered to a desired target (for
example, a protein, glycoprotein, lipoprotein, lipid, a
targeted cell, a targeted organ, a targeted organism or
other targeted moiety) and include antibodies,
preferably monoclonal antibodies, antibody fragments

20   such as Fab, Fab', $F(ab')_2$ fragments or any other
antibody fragments having the requisite target
specificity, hormones, biological response modifiers;
epitopes; chemical functionalities exhibiting target

25   specificity; and the like.

The antibodies or antibody fragments which may
be used in preferred starburst conjugates described
herein can be prepared by techniques well known in the

30   art. Highly specific monoclonal antibodies can be
produced by hybridization techniques well known in the
art, see, for example, Kohler and Milstein (1975,
Nature 256:495-497; and 1976, Eur. J. Immunol. 6:511-
519). Such antibodies normally have a highly specific

35   reactivity.

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In the antibody targeted starburst conjugates,
antibodies directed against any antigen or hapten may
be used. Although conventional polyclonal antibodies
may be used, monoclonal antibodies offer several
5   advantages. Each monoclonal antibody is highly
specific for a single epitope. In addition, large
amounts of each monoclonal antibody can be produced.
Antibodies used in the present invention may be
directed against, for example, tumors, bacteria, fungi,
10   viruses, parasites, mycoplasma, differentiation and
other cell membrane antigens, pathogen surface
antigens, toxins, enzymes, allergens, drugs and any
biologically active molecules. For a more complete
list of antigens see U. S. Patent 4,193,983.
15

It may be desirable to connect more antibodies
or fragments to the dendrimer, and in particular
instances to connect antibodies of different
specificities. For example, a bifunctional conjugate
20   which has the ability to localize and bind to a tumor
and then scavenge circulating cytotoxic, diagnostic, or
biostatic compounds can be designed.

In the absence of a target director (or in the
25   presence of a target director if desired), due to the
number of functional groups which can be located at or
near the surface of the dendrimer, all or a substantial
portion of such functional groups can be made anionic,
30   cationic, hydrophobic or hydrophilic to effectively aid
delivery of the starburst conjugate to a desired target
of the opposite charge or to a hydrophobic or
hydrophilic compatible target.

35   Preparation of the conjugates of formula (II)
using a P with a protected handle (S) is also intended

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as a process to prepare the conjugates of formula (II).
The reaction scheme is shown below:

5   S*P   loading   S*P*M   deprotection   P*M

                                    T*P*M   linking
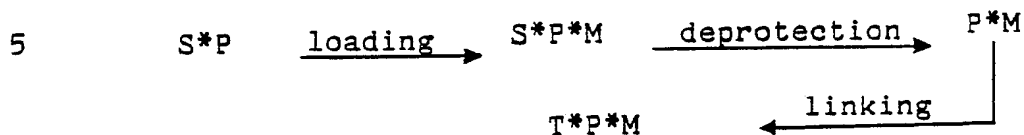
10 where

        S*P   represents the protected dendrimer;

        S*P*M   represents the protected dendrimer
                   conjugated with M;

15   P*M   represents the dendrimer conjugated
                   with M (starburst conjugate);

        T*P*M   represents the starburst conjugate
                   linked to the target director.

20
        Suitable solvents can be employed which do not
effect P*M. For example when S is t-butoxycarbamate, S
can be removed by aqueous acid.

25   Also preferred are starburst conjugates in
which the polymer is associated directly, or via
connectors; these starburst conjugates are represented
by the formula:

30          $[(T)_e - (C')_f]_g * (P)_x * [(C'')_h - (M)_y]_k$ (III)

wherein

each C' represents the same or different connecting
35 group;

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each C'' represents the same or different connecting
group;

g, and k each individually represent an integer of 1 or
5   greater;

f and h each individually represent an integer of 0 or
greater;

- indicates a covalent bond in instances where a
10   connecting group is present; and

P, x, *, M, y, T, and e are as previously defined
herein.

15 Preferred among the starburst conjugates of formula
(III) are those in which M is a radionuclide, drug,
toxin, signal generator, signal reflector or signal
absorber. Also preferred are those conjugates in which
x=1. Particularly preferred conjugates are those in
20   which x, e, f, h, and y are each 1, and g is one or
more and k is each individually 2 or more. Most
preferred are those conjugates in which x, e, f, h, y,
and g are each 1, and k is 2 or more. Also
25 particularly preferred are those starburst conjugates
in which M represents a bioactive agent such as a
radionuclide, drug, or toxin.

Suitable connecting groups which are
30   represented by C'' are groups which link the carried
pharmaceutical material to the dendrimer without
significantly impairing the effectiveness of the
carried pharmaceutical material or the effectiveness of
the target director(s) present in the starburst conju-
35 gate. These connectors must be stable (i.e., non-clea-
vable) or cleavable depending upon the mode of activity

of the carried pharmaceutical material and are
typically used in order to avoid steric hindrance
between the carried pharmaceutical material and the
polymer.

Most preferred are conjugates in which the
dendrimer is associated directly, or via connecting
group(s), to one antibody or antibody fragment. The
polymer in these preferred conjugates may, in addition,
be optionally associated either directly, or via
connecting group(s), to one or more other carried
materials, preferably a radioisotope. Such starburst
conjugates are represented by the formula:

$$[(\text{Antibody})_e - (C')_f]_g * (P)_x * [(C'')_h - (M)_y]_k \quad (IV)$$

wherein .

each Antibody represents an antibody or antibody
fragment capable of interacting with a desired epitope;

- indicates a covalent or coulombic bond in instances
where a connecting group is present; and

P, x, *, M, T, e, y, C', C'', g, k, f, and h are as
previously defined herein.

For the above synthesis of starburst dendrimers
(P) which have a functional group available for linking
(C' or C'') with a targeting director (T), the preferred
process requires that the reactive functionality be
protected as a synthetic precursor. This protection is
preferred because it enables the synthesis of dendrimer
or conjugates of very high quality. This process
allows for the chemical binding of a unit of carried

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pharmaceutical material (M) to the terminal functional
groups of the starburst dendrimer (P) in ways which
would otherwise result also in reaction with a linking
functional group, thus making it impossible to attach
5   to the targetting director (T). Subsequent
deprotection or synthetic conversion into the desired
linking functional group thus enables the starburst
conjugate to be linked to the targeting director.

10.   One of the preferred "functional groups for
linking" (hereafter referred to as a "handle") is an
aniline moiety. This group is preferred because it can
be used directly for linking to the targeting director,
or it can be readily modified to other functional
15   groups suitable for reaction with the targetting
director, e.g. isothiocyanate, isocyanate,
semithiocarbazide, semicarbazide, bromoacetamide,
iodoacetamide, and maleimide. The aniline moiety is
also preferred as a handle for linking with the
20   targetting directors because it can be readily
protected for use in starburst dendrimer synthesis, or
the nitro group can be used as a precursor which can be
converted into the desired amino function at the end of
25   the synthesis.

There are a number of protecting groups which
are suitable for protecting the anilino amino
functionality during starburst dendrimer synthesis.
30   (See Theodora W. Green, Protective Groups In Organic
Synthesis., Pub. John Wiley & Son, New York, 1981). A
preferred class of protecting groups are the carbamates
shown below.

35

-25-

5
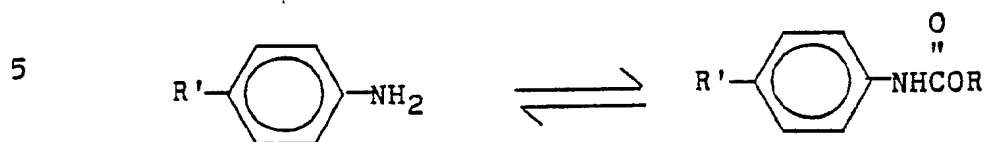
Many carbamates have been used for protection of
10 amines. The most preferred carbamates for starburst
dendrimer synthesis is the t-butoxycarbamate, R =
$-C(CH_3)_3$. Deprotection is achieved by mild acid
hydrolysis. Also preferred is the benzylcarbamate
15 protecting group, $R=-CH_2-$⬡ , which is preferred
when the dendrimer is susceptible to acid hydrolysis.
Deprotection is achieved by catalytic hydrogenation.
Also preferred is the 9-fluorenylmethylcarbamate,

20

$R=$ $-CH2-$
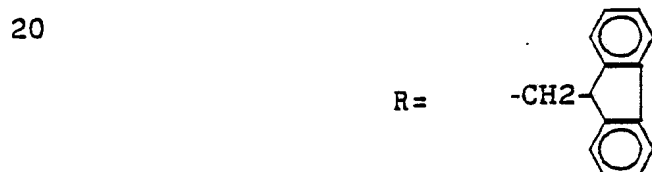
25   The phthalimide protecting group is also a
preferred example,

30
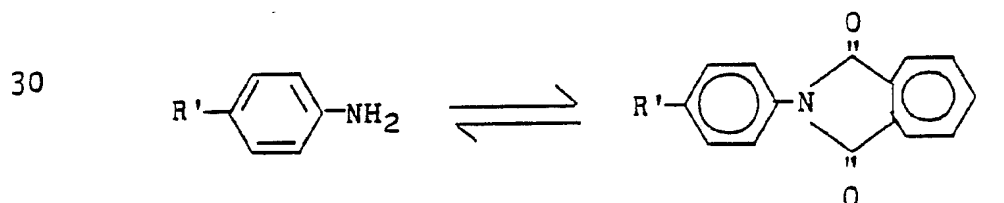
35

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Other protecting groups used for amines which
are well known in the literature could also be used in
this synthetic scheme. The above preferences are given
as illustrative examples only but are not the only
protecting groups which can be used. Any protecting
group which is stable under the reaction conditions and
can be removed without altering the integrity of the
starburst dendrimer can be employed.

The above process can introduce an aminophenyl
functionality into any agent containing an amino group
which is then conjugated with some bioactive agent,
e.g. monoclonal antibody or enzyme. The agent can be
conjugated by oxidative coupling to carbohydrates on
the bioactive agent, e.g. antibody. The aminophenyl
group also can be converted into an isothiocyanate or
isocyanate for subsequent reaction with the pendant
amino groups of lysine residues on the bioactive agent.

An alternate process involves the reaction of
an activated aryl halide, e.g. 4-nitrofluorobenzene,
with an amino-function; on the agent for conjugation,
e.g. starburst polyethyleneimines (PEI), and subsequent
catalytic hydrogenation of the nitro group to the
aniline functionality for subsequent conjugation. It
is particularly useful for agents, e.g. polyamines,
which need further modification prior to use, due to
the relative chemical inertness of the nitrophenyl
functionality to all non-reducing reaction conditions.
The more common bifunctional linking agents, e.g.
active esters or diisocyanates, which are reactive
under a large number of reaction conditions and which
would render them unusable for conjugation include:

5

10

; and

15

20
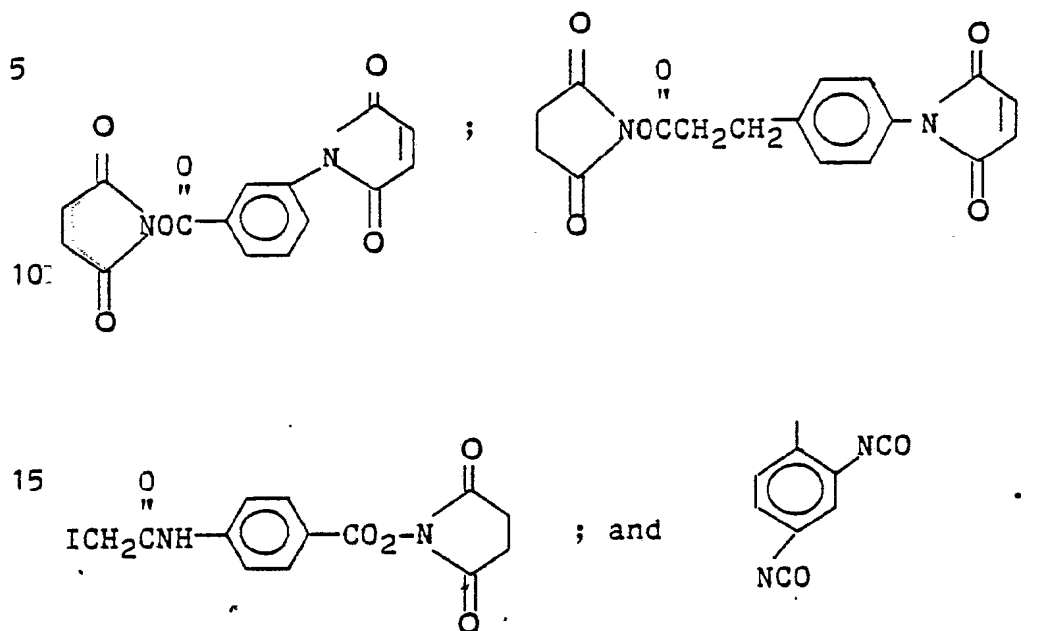
The invention also includes the use of nitro-substituted arylsulphonyl halides to give

25 sulphonamides,

e.g. O_2N—⟨phenyl⟩—SO_2X .

30
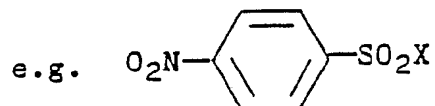
The advantage of this process over known processes of introducing an aminophenyl group for conjugation is that it takes place at a late stage of

35 the synthesis. Gansow et al., U.S. Patent 4,472,509, in his process introduced the nitrophenyl group at the

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first step of a long synthetic procedure, thereby
having limitations on the chemistry available.

This process also introduces a handle which is
5   clearly differentiable from the remainder of the
molecule. Manabe et al. disclosed that the ring
opening of succinic anhydride by residual amines gave a
coupling group through which conjugation to an antibody
was possible. This method however gave no means of
10 differentiating between any unchelated sites on the
polymer, since the chelating groups were the same as
the linking group.

The present process also provides for direct
15 synthesis of lanthanides with starburst dendrimers,
preferably by PEI acetate dendrimer. In contrast,
Denkewalter, U.S. Patent 4,289,872, states that just
putting acetates on the surface works. However, the
present reaction shows that PEI acetate, works much
20 better than PAMAM, i.e. surface of iminodiacetates is
only part of the story, the nature of the backbone, and
branching is very important as well. The PEI acetate
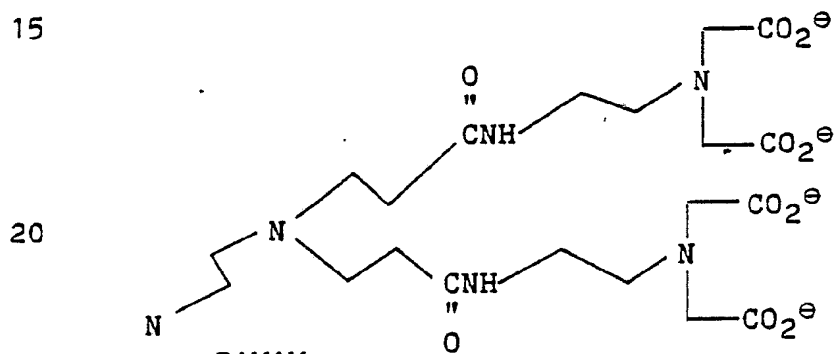has better chelating properties than the PAMAM acetate.

25

30

35

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5

PEI

15

20
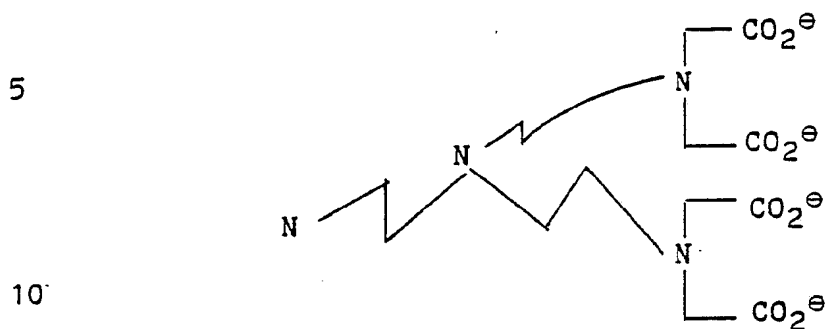
PAMAM

25   Preferred among the starburst conjugates of
formula (IV) are those in which M is a radionuclide,
drug, toxin, signal generator, signal reflector or
signal absorber. Also preferred are those conjugates
30 in which x=1. Particularly preferred are those
conjugates in which x, e, f, h, and y are each 1, and g
is one or more and k is each individually 2 or more.
Most preferred are those conjugates in which x, e, f,
h, y, and g are each 1, and k is 2 or more. Also
35 particularly preferred are those starburst conjugates
in which "Antibody" represents a monoclonal antibody or

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an epitope binding fragment thereof; and especially
preferred are those in which M represents a bioactive
agent such as a radionuclide, drug, or toxin.

5   The starburst conjugates can be used for a
variety of _in vitro_ or _in vivo_ diagnostic applications
such as radioimmunoassays, electron microscopy, enzyme
linked immunosorbent assays, nuclear magnetic resonance
spectroscopy, contrast imaging, and immunoscintography,
10   in analytical applications, in therapeutic applications
as a carrier of antibiotics, radionuclides, drugs or
other agents suitable for use in the treatment of
disease states such as cancer, autoimmune diseases,
central nervous system disorders, infectious diseases,
15   and cardiac disorders, or used as starting materials
for making other useful agents.

       The present invention is also directed to
starburst conjugate compositions in which the starburst
20   conjugates are formulated with other suitable vehicles.
The starburst conjugate compositions may optionally
contain other active ingredients, additives and/or
diluents.

25
       The preferred starburst polymer for use in the
starburst conjugates of the present invention is a
polymer that can be described as a starburst polymer
having at least one branch (hereinafter called a core
30   branch), preferably two or more branches, emanating
from a core, said branch having at least one terminal
group provided that (1) the ratio of terminal groups to
the core branches is more than one, preferably two or
greater, (2) the density of terminal groups per unit
35   volume in the polymer is at least 1.5 times that of an
extended conventional star polymer having similar core

and monomeric moieties and a comparable molecular
weight and number of core branches, each of such
branches of the extended conventional star polymer
bearing only one terminal group, and (3) a molecular
5   volume that is no more than about 80 percent of the
molecular volume of said extended conventional star
polymer as determined by dimensional studies using
scaled Corey-Pauling molecular models. As used herein,
the term "dense" as it modifies "star polymer" or
10   "dendrimer" means that it has a smaller molecular
volume than an extended conventional star polymer
having the same molecular weight. The extended
conventional star polymer which is used as the base for
comparison with the dense star polymer is one that has
15   the same molecular weight, same core and monomeric
components and same number of core branches as the
dense star polymer. By "extended" it is meant that the
individual branches of the conventional star polymer
are extended or stretched to their maximum length,
20   e.g., as such branches exist when the star polymer is
completely solvated in an ideal solvent for the star
polymer. In addition while the number of terminal
groups is greater for the dense star polymer molecule
than in the conventional star polymer molecule, the
25   chemical structure of the terminal groups is the same.

Dendrimers used in the conjugates of the
present invention can be prepared by processes known in
30   the art. The above dendrimers, the various coreactants
and core compounds, and process for their preparation
can be as defined in U. S. Patent 4,587,329.

The dendrimers, for use in the conjugates of
35   the present invention, can have terminal groups which
are sufficiently reactive to undergo addition or

substitution reactions. Examples of such terminal
groups include amino, hydroxy, mercapto, carboxy,
alkenyl, allyl, vinyl, amido, halo, urea, oxiranyl,
aziridinyl, oxazolinyl, imidazolinyl, sulfonato,
5 phosphonato, isocyanato and isothiocyanato. The
terminal groups can be modified to make them
biologically inert, for example, to make them non-
immunogenic or to avoid non-specifc uptake in the
liver, spleen or other organ. The dendrimers differ
10 from conventional star or star-branched polymers in
that the dendrimers have a greater concentration of
terminal groups per unit of molecular volume than do
conventional extended star polymers having an
equivalent number of core branches and an equivalent
15 core branch length. Thus, the density of terminal
groups per unit volume in the dendrimer usually is at
least 1.5 times the density of terminal groups in the
conventional extended star polymer, preferably at least
20 5 times, more preferably at least 10 times, most
preferably from 15 to 50 times. The ratio of
terminal groups per core branch in the dense polymer is
preferably at least 2, more preferably at least 3, most
preferably from 4 to 1024. Preferably, for a given
25 polymer molecular weight, the molecular volume of the
dense star polymer is less than 70 volume percent, more
preferably from 16 to 60, most preferably from 7 to
50 volume percent of the molecular volume of the
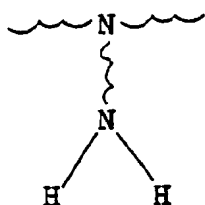30 conventional extended star polymer.

Preferred dendrimers for use in the conjugates
of the present invention are characterized as having a
univalent or polyvalent core that is covalently bonded
35 to dendritic branches. Such ordered branching can be

illustrated by the following sequence wherein G
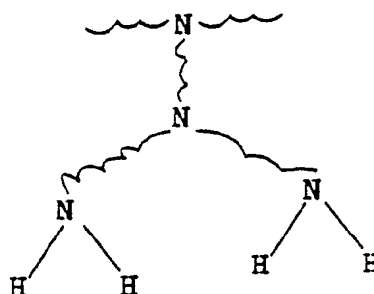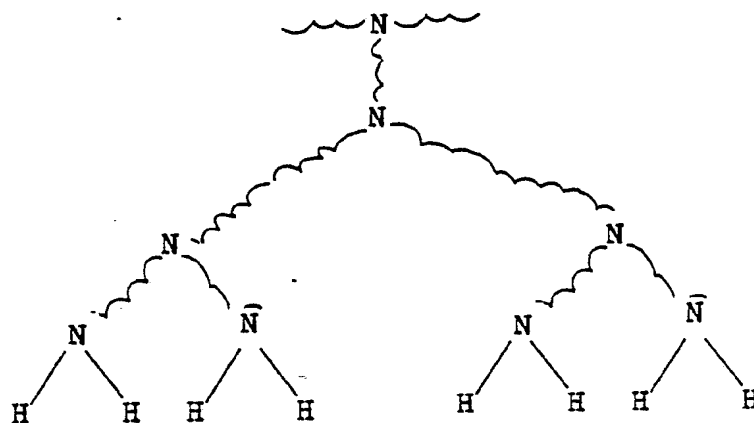indicates the number of generations:

G = 1

G = 2

G = 3

Mathematically, the relationship between the
number (#) of terminal groups on a dendritic branch and
the number of generations of the branch can be
represented as follows:

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$$\text{\# of terminal groups per dendritic branch} = \frac{N_r{}^G}{2}$$

wherein G is the number of generations and N_r is the
repeating unit multiplicity which is at least 2 as in
the case of amines. The total number of terminal
groups in the dendrimer is determined by the following:

$$\text{\# of terminal groups per dendrimer} = \frac{N_c N_r{}^G}{2}$$

wherein G and N_r are as defined before and N_c
represents the valency (often called core
functionality) of the core compound. Accordingly, the
dendrimers of this invention can be represented in its
component parts as follows:

$$(\text{Core})\left\{(\text{Repeat Unit})_{\frac{N_r{}^{G}-1}{N_r-1}} \quad \text{Terminal Moiety} \quad N_r{}^G \right\}_{N_c}$$
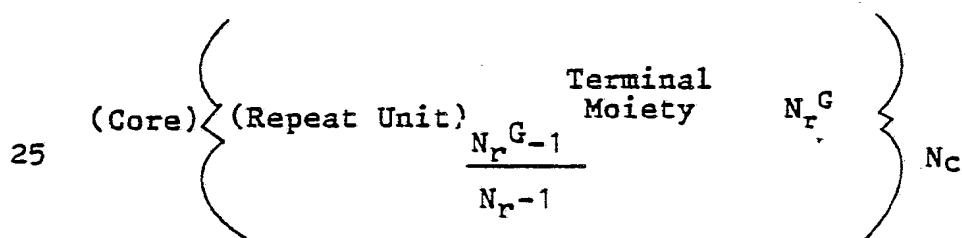
wherein the Core, Terminal Moiety, G and N_c are as
defined before and the Repeat Unit has a valency or
functionality of N_r + 1 wherein N_r is as defined
before.

A copolymeric dendrimer which is a preferred
dendrimer for the purposes of this invention is a
unique compound constructed of polyfunctional monomer

units in a highly branched (dendritic) array. The
dendrimer molecule is prepared from a polyfunctional
initiator unit (core compound), polyfunctional
repeating units and terminal units which may be the
same or different from the repeating units. The core
compound is represented by the formula $\text{(I)}\ (Z^C)_{Nc}$
wherein (I) represents the core, Z^C represents the
functional groups bonded to (I) and Nc represents the
core functionality which is preferably 2 or more, most
preferably 3 or more. Thus, the dendrimer molecule
comprises a polyfunctional core, (I) bonded to a number
(N_c) of functional groups, Z^C, each of which is
connected to the monofunctional tail of a repeating
unit, $X^1Y^1(Z^1)_{N^1}$, of the first generation and each of
the Z groups of the repeating unit of one generation is
bonded to a monofunctional tail of a repeating unit of
the next generation until the terminal generation is
reached.

In the dendrimer molecule, the repeating units
are the same within a single generation, but may differ
from generation to generation. In the repeating unit,
$X^1Y^1(Z^1)_{N^1}$, X^1 represents the monofunctional tail of
the first generation repeating unit, Y^1 represents the
moiety constituting the first generation, Z^1 represents
the functional group of the polyfunctional head of the
repeating unit of the first generation and may be the
same as or different from the functional groups of the
core compound, $\text{(I)}(Z^C)_{Nc}$, or other generations; and N^1
is a number of 2 or more, most preferably 2, 3 or 4,
which represents the multiplicity of the polyfunctional
head of the repeating unit in the first generation.
Generically, the repeating unit is represented by the
formula $X^iY^i(Z^i)_{N^i}$ wherein "i" represents the

particular generation from the first to the t-1
generation. Thus, in the preferred dendrimer molecule,
each Z^1 of the first generation repeating unit is
connected to an X^2 of a repeating unit of the second
generation and so on through the generations such that
each Z^i group for a repeating unit $X^i Y^i (Z^i)_{N^i}$ in
generation number "i" is connected to the tail (X^{i+1})
of the repeating unit of the generation number "i+1".
The final or terminal of a preferred dendrimer molecule
comprises terminal units, $X^t Y^t (Z^t)_{N^t}$ wherein t
represents terminal generation and X^t, Y^t, Z^t and N^t
may be the same as or different from X^i, Y^i, Z^i and N^i
except that there is no succeeding generation connected
to the Z^t groups and N^t may be less than two, e.g.,
zero or one. Therefore the preferred dendrimer has a
molecular formula represented by

$$\left(\textcircled{I}(Z^c)_{N_c} \right) \left\{ \left(X^i \ Y^i \ (Z)^i_{N^i} \right)^{i-1}_{N_c \pi N^n}_{\substack{n \text{ is } 1}} \right\} \left(X^t Y^t (Z^t)_{N^t} \right)^{t-1}_{N_c \pi N^n}_{\substack{n \text{ is } 1}}$$

where i is 1 to t-1

wherein the symbols are as previously defined. The π
function is the product of all the values between its
defined limits. Thus

$$\prod_{n=1}^{i-1} N^n = (N^1)(N^2)(N^3)\cdots(N^{i-2})(N^{i-1})$$

which is the number of repeat units, $X^i Y^i (Z^i)_{N^i}$,
comprising the ith generation of one dendritic branch
and when i is 1, then

5

$$\prod_{n=1}^{0} = 1 \quad .$$

10:

In copolymeric dendrimers, the repeat unit for one
generation differs from the repeat unit in at least one
other generation. The preferred dendrimers are very
symmetrical as illustrated in structural formulas
15 described hereinafter. Preferred dendrimers may be
converted to functionalized dendrimers by contact with
another reagent. For example, conversion of hydroxyl
in the terminal generation to ester by reaction with an
acid chloride gives an ester terminally functionalized
20 dendrimer. This functionalization need not be carried
out to the theoretical maximum as defined by the number
of available functional groups and, thus, a
functionalized dendrimer may not have high symmetry or
25 a precisely defined molecular formula as is the case
with the preferred dendrimer.

In a homopolymeric dendrimer, all of the repeat
units, $X^i Y^i (Z^i)_{N^i}$, are identical. Since the values of
30 all N^i are equal (defined as N_r), the product function
representing the number of repeat units reduces to a
simple exponential form. Therefore, the molecular
formula may be expressed in simpler form as:

35

5
$$\left(\textcircled{I}(Z^c)_{N_c}\right)\left\{\left(X^i\ Y^i\ (Z^i)_{N^i}\right)_{N_cN_r^{i-1}}\right\}\left(X^tY^t(Z^t)_{N^t}\right)_{N_cN_r^{(t-1)}}$$

10   where $i = 1$ to $t-1$

This form still shows the distinction between
15 the different generations i, which each consist of
$N_cN_r^{(i-1)}$ repeating units, $X^iY^i(Z^i)_{N^i}$. Combining the
generations into one term gives:

$$\left(\textcircled{I}(Z^c)_{N_c}\right)\left(X^rY^r(Z^r)_{N_r}\right)_{N_c\frac{N_r^{(t-1)}-1}{N_r-1}}\left(X^tY^t(Z^t)_{N^t}\right)_{N_cN_r^{(t-1)}}$$

or

$$\left(\textcircled{I}(Z^c)_{N_c}\right)\left\{(X^rY^r(Z^r)_{N_r})_{\frac{N_r^{(t-1)}-1}{N_r-1}}\left(X^tY^t(Z^t)_{N^t}\right)_{N_r^{(t-1)}}\right\}_{N_c}$$

Core   Repeat Unit   Terminal Unit

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wherein $X^r Y^r (Z^r)_{Nr}$ is the repeating unit which is used
in all generations i.

Consequently, if a polymer compound will fit
into these above formulae, then the polymer is a
starburst polymer. Conversely, if a polymer compound
will not fit into these above formulae, then the
polymer is not a starburst polymer. Also, to determine
whether a polymer is a starburst polymer, it is not
necessary to know the process by which it was prepared,
but only whether it fits the formulae. The formulae
also demonstrate the generations (G) or tiering of
dendrimers.

Clearly, there are several ways to determine
the ratio of agent (M) to dendrimer (P) which depend
upon how and where the association of P*M occurs. When
there is interior encapsulation, the weight ratio of
M:P usually is 10:1, preferably 8:1, more preferably
5:1, most preferably 3:1. The ratio can be as low as
0.5:1 to 0.1:1. When interior stoichiometry is used,
the weight ratio of M:P is the same as for interior
encapsulation. When exterior stoichiometry is
determined, the mole/mole ratio of M:P is given by the
following formulae:

	M	:	P
(A)	$5\ N_c N_t N_r^{G-1}$		1
(B)	$3\ N_c N_t N_r^{G-1}$		1
(C)	$1\ N_c N_t N_r^{G-1}$		1

where N_c means the core multiplicity, N_t means the
terminal group multiplicity, and N_r means branch

-40-

juncture multiplicity. The $N_cN_tN_rG^{-1}$ term will result
in the number of Z groups. Thus, for example, (A)
above may result when proteins, enzymes or highly
charged molecules are on the surface; (B) above when it
5   is aspirin or octanoic acid; (C) above when converting
surface ester groups to carboxylate ions or groups.

Of course other structures of various
dimensions can be readily prepared by one skilled in
10   the art by appropriately varying the dendrimer
components and number of generations employed. A
roughly scaled comparison of three different dendrimer
series relative to an IgG antibody is seen in Figure 3.
The series of drawings indicated by Figure 3(B) I shows
15   the starburst polyamidoamines (PAMAM); by II shows the
starburst polyethers (PE); and by III shows the
starburst polyethyleneimines (PEI). In a manner
similar to that of Figure 1, all three series (I, II
20   and III) have their far left drawing showing the
initiator core, the next drawing from the left showing
the starbranch oligomer, and the remaining drawings
showing the starburst oligomers and respective
starburst bridged dendrimers. It can be seen that in
25   these series of scale drawings that the dendrimer
dimensions are close to those noted for the IgG
antibody Figure 3(A). The IgG antibody is shown to the
far left in Figure 3. The scale is 1 mm = 3.5Å. In
Figure 3(A) the variable region is shown by (A); the
30   constant region by (B); and the carbohydrate attachment
sites by (C). Approximate measurements shown on Figure
3 are (1) is 35-40Å; (2) is 70Å; and (3) is 60Å.
These dimensional properties are preferred for instance
35   where targeting involves exiting from the vascular
system. Therefore dendrimer diameters of 125 Angstrom

units or less are particularly preferred in that they
may allow exiting from the vascular system to targeted
organs serviced by continuous or fenestrated
capillaries. These dimensions are significant in that
5   they are small compared to the size of a potential
targeting component such as an antibody (see Figure 3).
A linear polymer of comparable molecular weight would
have a radius of gyration; (in its fully extended
form), that would be much larger than the same
10 molecular weight dendrimer. A linear polymer of this
type would be expected to adversely affect the
molecular recognition properties of many accepted
targeting components. It is also desirable that the
conjugates be of minimum molecular volume so as not to
15 discourage, extravasation, e.g., by coupling Fab, Fab'
or other appropriate antibody fragment to low molecular
volume dendrimers.

20   Dendrimers are desirable for the delivery of
radionuclides or strongly paramagnetic metal ions to
tumor sites because of their ability to chelate a
number of metal ions in a small volume of space.
Coupling to antibodies or antibody fragments which are
25 specific for tumors may deliver a number of metals per
antibody, with only a single modification to the
antibody.

        Linking target directors to dendrimers is
30 another aspect of the present invention. In preferred
embodiments of the present invention, particularly
where it is desired to use an antibody as a target
director, a reactive functional group such as a
carboxyl, sulfhydryl, reactive aldehyde, reactive
35 olefinic derivative, isothiocyanato, isocyanato, amino,
reactive aryl halide, or reactive alkyl halide can

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conveniently be employed on the dendrimer. The reactive functional groups can be introduced to the dendrimer using known techniques, for example:

5   (1) Use of a heterofunctional initiator (as a starting material for synthesizing the dendrimer) which has incorporated into it functional groups of different reactivity. In such heterofunctional initiator at least one of the functional groups will serve as an
10   initiation site for dendrimer formation and at least one of the other functional groups will be available for linking to a target director but unable to initiate dendrimer synthesis. For example, use of protected aniline to allow further modification of NH_2 groups
15   within the molecule without reacting the aniline NH_2.

The functional group which will be available for linking to a target director may be part of the initiator molecule in any one of three forms; namely:
20

(a) In the from in which it will be used for linking with the target director. This is possible when none of the synthetic steps involved in the dendrimer synthesis can
25   result in reaction at this center.

(b) When the functional group used for linking to the targeting director is reactive in the synthetic steps involved in the
30   dendrimer synthesis, it can be protected by use of a protecting group, which renders the group unreactive to the synthetic procedures involved, but can itself be readily removed in a manner
35

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which does not alter the integrity of the
remainder of the macromolecule.

5   (c)   In the event that no simple protecting
group can be formed for the reactive
functionality to be used for linking with
the targeting director, a synthetic
precursor can be used which is unreactive
in all the synthetic procedures used in
10   the dendrimer synthesis. On completion of
the synthesis, this functional group must
be readily convertible into the desired
linking group in a manner which does not
alter the integrity of the remainder of
15   the molecule.

. (2) Coupling (covalently) the desired reactive
functional group onto a preformed dendrimer, the
reagent used must contain a functionality which is
20 readily reacted with the terminal functional groups of
the dendrimer. The functional group to be ultimately
used to link with the targetting agent can be in its
final form, as a protected functionality, or as a
synthetic precursor. The form in which this linking
25 functionality is used depends on its integrity during
the synthetic procedure to be utilized, and the ability
of the final macromolecule to withstand any conditions
necessary to make this group available for linking.
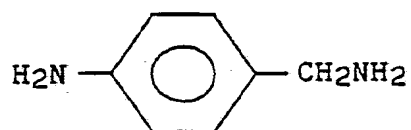30 For example, the preferred route for PEI uses

F—⟨◯⟩—NO2
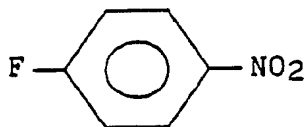
35

-44-

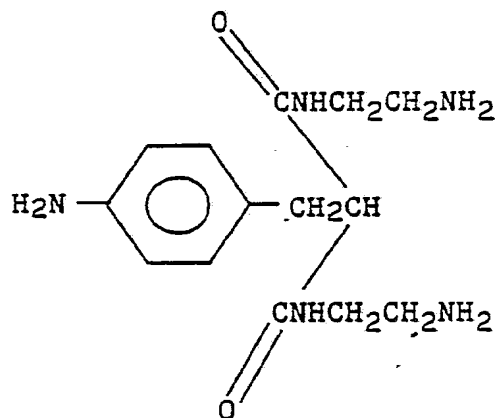Examples of heterofunctional initiators for use in (1) above, include the following illustrative examples:

5

$$H_2N - \bigcirc - CH_2NH_2 \qquad ;$$

10

15

$$H_2N - \bigcirc - \underset{\underset{\displaystyle CNHCH_2CH_2NH_2}{\parallel}}{\overset{\underset{\displaystyle CNHCH_2CH_2NH_2}{\parallel}}{\overset{O}{}}} CH_2CH \qquad ;$$

20

25

$$H_2N - \bigcirc - CH_2\underset{\underset{\displaystyle CH_2NH_2}{\mid}}{\overset{\overset{\displaystyle NH_2}{\mid}}{CH}} \qquad ;$$

30

35

5

$$(CH_3)_3COCNH \underset{\underset{O}{\parallel}}{} \text{—} \bigcirc \text{—} CH_2CH \begin{array}{c} \overset{\overset{O}{\parallel}}{C}NHCH_2CH_2NH_2 \\ \\ \underset{\underset{O}{\parallel}}{C}NHCH_2CH_2NH_2 \end{array} \quad ;$$

10

15

$$(CH_3)_3COCNH \underset{\underset{O}{\parallel}}{} \text{—} \bigcirc \text{—} CH_2CH \begin{array}{c} CH_2NH_2 \\ \\ CH_2NH_2 \end{array} \quad ;$$

20

$$H_2N \text{—} \bigcirc \text{—} CH_2CH_2NH_2 \quad ;$$

25

30

35

5   $H_2N-\bigcirc-CH_2CH\begin{smallmatrix}CH_2NH_2\\[1mm]CH_2NH_2\end{smallmatrix}$;

10

    $O_2N-\bigcirc-CH_2CH_2NH_2$;

15

20

    $O_2N-\bigcirc-CH_2CH\begin{smallmatrix}CH_2NH_2\\[1mm]CH_2NH_2\end{smallmatrix}$; and

25

30

35

$$O_2N - \langle \bigcirc \rangle - CH_2CH \begin{array}{c} CH_2CH_2NH_2 \\ | \\ CH_2NCH_2CH_2NH_2 \\ | \\ CH_2NCH_2CH_2NH_2 \\ | \\ CH_2CH_2NH_2 \end{array}$$
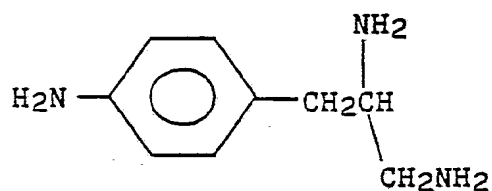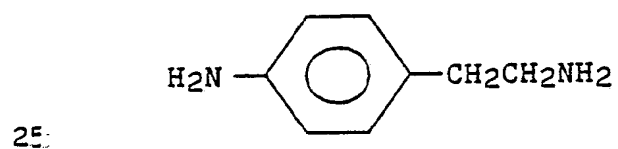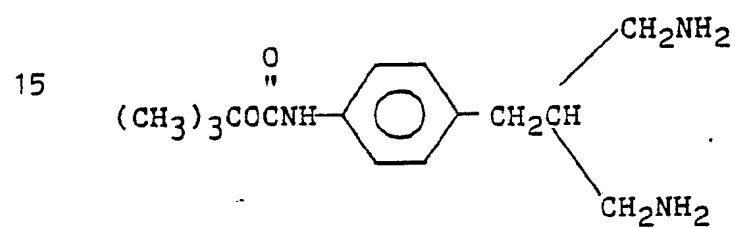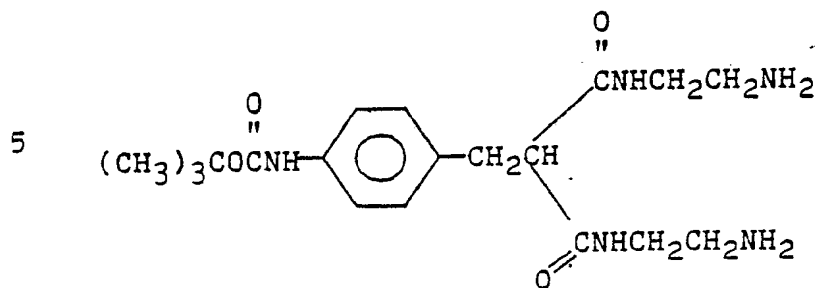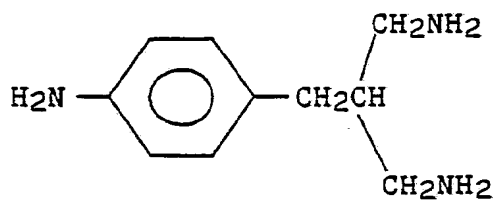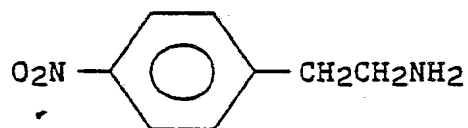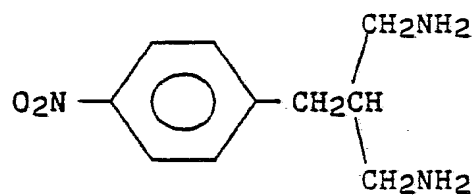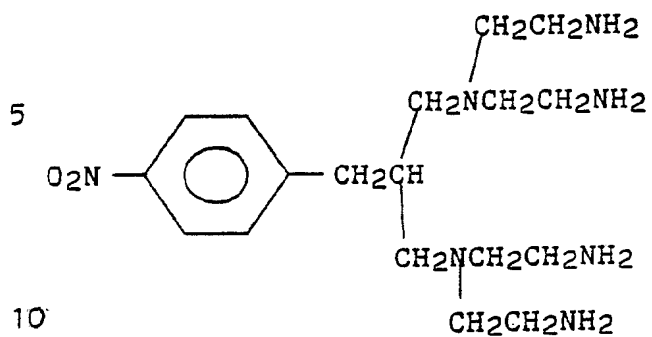
There are several chemistries of particular importance:

1) Starburst Polyamidoamides ("PAMAM") Chemistry;

2) Starburst Polyethyleneimines ("PEI") Chemistry;

3) Starburst PEI compound with a surface of PAMAM;

4) Starburst polyether ("PE") chemistry.

Modifications of the dendrimer surface
functionalities may provide other useful functional
groups such as the following:

5
$-OPO_3H_2$, $-PO_3H_2$, $-PO_3H^{(-1)}$, $-PO_3^{(-2)}$, $-CO_2^{(-1)}$, $-SO_2H$,
$-SO_2^{(-1)}$, $-SO_3H$, $-SO_3^{(-1)}$, $-NR^1R^2$, $-R^5$, $-OH$, $-OR^1$, -
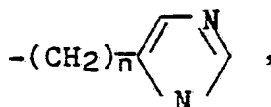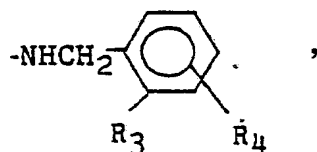NH_2, perfluorinated alkyl, $-\underset{O}{\overset{\parallel}{C}}NHR^1$, $-\underset{O}{\overset{\parallel}{C}}OH$,

10

$-(CH_2)_n$⟨pyridine⟩ , $-N=CH$⟨benzene R_3 R_4⟩ ,

1

$-NHCH_2$⟨benzene R_3 R_4⟩ , $-(CH_2)_n$⟨pyrazine N N⟩ , $-(CH_2)_n$⟨pyridine N⟩ ,

2

wherein
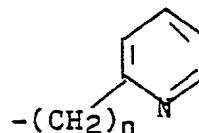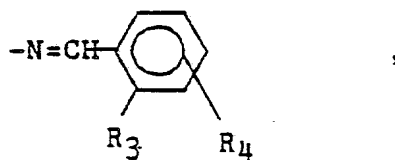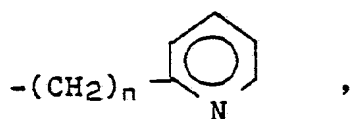
R represents alkyl, aryl or hydrogen;

R^1 represents alkyl, aryl, hydrogen, or

25

$$-N\underset{(CH_2)n}{\overset{(CH_2)n}{\diagup\diagdown}}X \quad ;$$
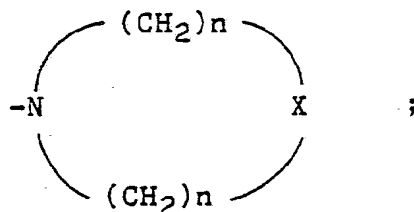
30

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R2 represents alkyl, aryl, or

$$-N \left\langle \begin{array}{c} (CH_2)n \\ (CH_2)n \end{array} \right\rangle X \quad ;$$
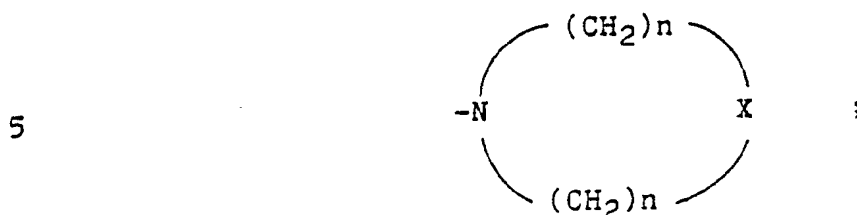
R3 represents -OH, -SH, -CO2H, -SO2H, or -SO3H;

R4 represents alkyl, aryl, alkoxy, hydroxyl, mercapto, carboxyl, nitro, hydrogen, bromo, chloro, iodo, or fluoro;

R5 represents alkyl;

X represents NR, O or S; and

n represents the integer 1, 2 or 3;

polyethers; or other immuno insensitive moieties..

The choice of functional group depends upon the particular end use for which the dendrimer is designed.

Linking of antibodies to dendrimers is another aspect of the present invention. Typically, the antibodies or antibody fragments are linked to the dendrimer by techniques well known in the art such as attachment between a functional group on the dendrimer and moieties such as carbohydrate, amino, carboxyl, or sulfhydryl functionalities present in the antibody or antibody fragment. In some instances connecting groups may be used as connectors or spacers between the dendrimer and antibody or antibody fragment. The attachment of the dendrimer to the antibody or antibody fragment should be performed in a manner which does not significantly interfere with the immunoreactivity of

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the antibody or antibody fragment, that is, by binding
the antibody or antibody fragment via a functionality
in the antibody or antibody fragment which is not a
part of the antigen recognition and binding site.

5   The following examples further illustrate the
invention but are not to be construed as a limitation
on the scope of the invention. The lettered examples
concern the preparation of starting materials; the
10   numbered examples concern the preparation of products.

<u>Example A:</u> Preparation of 2-Carboxamido-3-(4'-nitro-
phenyl)-propanamide.

15   p-Nitrobenzyl malonate diethylester (2.4 grams
(g), 8.13 mmole) was dissolved in 35 ml of methanol.
The solution was heated to 50-55°C with stirring and a
stream of anhydrous ammonia was bubbled through the
solution for 64 hours. The solution was cooled and the
20   white, flocculant product was filtered and
recrystallized from 225 milliliters (ml) of boiling
methanol to afford 1.85 g (7.80 mmole) of bis amide in
96% yield (mp = 235.6°C(d)).

25   The structure was confirmed by MS, ^{1}H and ^{13}C NMR
spectroscopy.

<u>Anal:</u> Calc. for $C_{10}H_{11}O_4N_3$

30

	<u>C</u>	<u>H</u>	<u>N</u>
Theo:	50.63	4.69	17.72
Found:	50.75	4.81	17.94

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Example B: Preparation of 1-Amino-2-(aminomethyl)-3-
(4'-nitrophenyl)propane.

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       2-Carboxamido-3-(4'nitrophenyl)propanamide (2.0
g, 8.43 mmole) was slurried in 35 ml of dry tetrahydro-
furan under a nitrogen atmosphere with stirring. To
this mixture was added borane/tetrahydrofuran complex
(106 ml, 106 mmole) via syringe. The reaction mixture
was then heated to reflux for 48 hours during which
time the suspended amide dissolved. The solution was
cooled and the tetrahydrofuran was removed in vacuo
using a rotary evaporator. The crude product and
borane residue was dissolved in 50 ml of ethanol and
this solution was purged with anhydrous hydrogen
chloride gas. The solution was refluxed for 1 hour and
the solvent removed in vacuo. The crude hydrochloride
salt was dissolved in 15 ml of deionized water and
extracted with two 50 ml portions of methylene
chloride. The aqueous layer was cooled in an ice bath
under an argon blanket and 50% sodium hydroxide was
slowly added until basic pH=11.7. The basic aqueous
layer was extracted with four 25 ml portions of
methylene chloride and these combined extracts were
evaporated (rotary) to give 1.45 g of amber colored
oil. This oil was triturated with diethyl ether (50
ml) and filtered under pressure through a short silica
gel (grade 62 Aldrich) column. The column was washed
with 100 ml of ether and the combined filtrates were
vacuum evaporated giving 1.05 g (5.02 mmole) of the
titled diamine as a clear oil (mp = 275-278°C(d) bis HCl
salt).

The structure was confirmed by MS, ^{1}H and ^{13}C NMR
spectroscopy.

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<u>Anal:</u> Calc. for $C_{10}H_{17}N_3O_2Cl_2$

	C	H	N
Theo:	42.57	6.07	14.89
Found:	43.00	6.14	15.31

5   <u>Example C:</u> Preparation of 1-Amino-2-(aminomethyl)-3-(4'-aminophenyl)propane.

     Borane/tetrahydrofuran solution (70 ml, 70 mmole) was added under nitrogen <u>via</u> a cannula needle to
10   a flask containing 4-amino-benzyl malonamide (1.5 g, 7.24 mmole) with stirring. The solution was brought to reflux for 40 hours. The colorless solution was cooled and excess tetrahydrofuran was removed by rotary evaporation leaving a clear gelatinous oil. Methanol
15   (50 ml) was cautiously added to the oil with notable gas evolution. Dry hydrogen·chloride was bubbled through the suspension to effect dissolution and the solution was then refluxed for 1 minute. The methanol/HCl was rotary evaporated and the resulting
20   hydrochloride salt was carried through the same dissolution/reflux procedure again. The hydrochloride salt obtained was dissolved in 10 ml of water and cooled in an ice bath under argon. Concentrated sodium
25   hydroxide (50%) was added slowly with stirring to pH=11. The aqueous portion was then extracted with 2 X 100 ml portions of chloroform which were combined and filtered through a short silica gel plug without drying. The solvent was removed <u>in vacuo</u> (rotary)
30   affording the title compound (0.90 g, 5.02 mmole) in 70% yield (R_f=0.65 - $CHCl_3$/MeOH/NH_4OH conc - 2/2/1). The structure was confirmed by ^{1}H and ^{13}C NMR and used without further purification.

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Example D: Preparation of 6-(4-Aminobenzyl)-1,4,8,11-
tetraaza-5,7-dioxoundecane.

5

10.

15

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4-Aminobenzyl malonate dimethylester (2.03 g,
8.43 mmole) was dissolved in 10 ml of methanol. This
solution was added dropwise to a stirred solution of
freshly distilled ethylene diamine (6.00 g, 103.4
mmole) in 10 ml of methanol under nitrogen over a 2
hour period. The clear solution was stirred for 4 days
and Thin Layer Chromotography (TLC) analysis indicated
total conversion of diester (Rf = 0.91) to the bis
amide (Rf = 0.42 - 20% conc NH4OH/80% ethanol). This
material was strongly ninhydrin positive. The methanol
and excess diamine were removed on a rotary evaporator
and the resulting white solid was vacuum dried (10^{-1}
mm, 50°C) overnight to afford crude product (2.45g, 8.36
mmole) in 99% yield. An analytical sample was
recrystallized from chloroform/hexane, MP = 160-161°C.
The mass spectral, 1H and 13C NMR data were consistent
with the proposed structure.

Example E: Reaction of Mesyl Aziridine with 1-Amino-2-
(aminomethyl)-3-(4-nitrophenyl)propane.

25

30.

35

1-Amino-2-(aminomethyl)-3-(4-nitrophenyl)-
propane (400 mg, 1.91 mmole, >96% pure) was dissolved
in 10.5 ml of absolute ethanol under nitrogen. Mesyl
aziridine (950 mg, 7.85 mmole) was added to the stirred
diamine solution as a solid. The reaction was stirred
at 25°C for 14 hours using a magnetic stirrer and during
this period a white, gummy residue formed on the sides
of the flask. The ethanol was decanted and the residue
was triturated with another 15 ml portion of ethanol to
remove any unreacted aziridine. The gummy product was

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vacuum dried (10^{1}mm, 25°C) to afford the tetrakis methyl
sulfonamide (1.0 g, 1.44 mmole) in 75% yield (Rf = 0.74
- NH$_4$OH/ethanol - 20/80). The structure was confirmed
by ^{1}H and ^{13}C nuclear magnetic resonance (NMR)
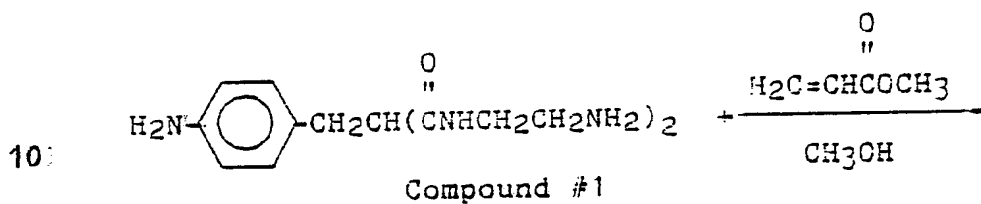5   spectroscopy.

Example F: Preparation of 2-(4-Nitrobenzyl)-1,3-(bis-
N,N-2-aminoethyl)diaminopropane.

10.   The crude methylsulfonamide (650 mg, 0.94
mmole) was dissolved in 5 ml of nitrogen purged,
concentrated sulfuric acid (98%). This solution was
maintained under nitrogen and heated to 143-146°C for 27
minutes with vigorous stirring. A slight darkening was
15   noted and the cooled solution was poured into a stirred
solution of ether (60 ml). The precipitated white salt
cake was filtered and immediately dissolved in 10 ml of
deionized water. The pH of the solution was adjusted
to pH=11 with 50% NaOH under argon with cooling. The
20   resulting solution was mixed with 90 ml of ethanol and
the precipitated inorganic salts were filtered. The
solvent was removed from the crude amine under reduced
pressure and to the resulting light brown oil was added
25   190 ml of toluene under nitrogen. The mixture was
stirred vigorously and water was removed through
azeotropic distillation (Dean-Stark trap) until the
remaining toluene acquired a light yellow color (30-40
ml remaining in pot). The toluene was cooled and
30   decanted from the dark, intractable residues and salt.
This solution was stripped of solvent in vacuo and the
resulting light yellow oil was vacuum dried (0.2 mm,
35°C) overnight affording 210 mg of the product (60%)
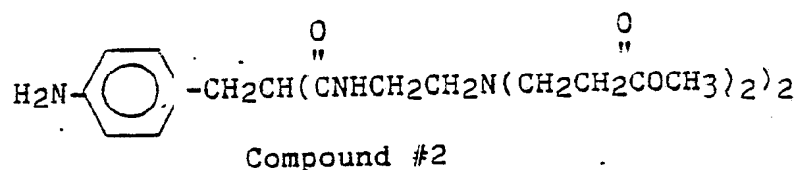which was characterized by MS, ^{1}H and ^{13}C NMR.
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Example G: Preparation of a starburst polymer
(containing an aniline derivative) of one half
generation represented by the following scheme:

5

10

$$H_2N-\bigcirc-CH_2CH(\overset{O}{\overset{\|}{C}}NHCH_2CH_2NH_2)_2 \quad + \quad \frac{H_2C=CHCOCH_3}{CH_3OH}$$

Compound #1

15

$$H_2N-\bigcirc-CH_2CH(\overset{O}{\overset{\|}{C}}NHCH_2CH_2N(CH_2CH_2\overset{O}{\overset{\|}{C}}OCH_3)_2)_2$$

Compound #2

20

Methyl acrylate (2.09 g, 24 mmole) was
dissolved in methanol (15 ml). The compound 6-(4-
aminobenzyl)-1,4,8,11-tetraaza-5,7-dioxoundecane (1.1
g, 3.8 mmole) (i.e., Compound #1) was dissolved in
25 methanol (10 ml) and was added slowly over 2 hours with
rigorous stirring to the methyl acrylate solution. The
reaction mixture was stirred for 48 hours at ambient
temperatures. The solvent was removed on the rotary
evaporator maintaining the temperature below 40°C. The
30 ester (Compound #2) was obtained as a yellow oil (2.6
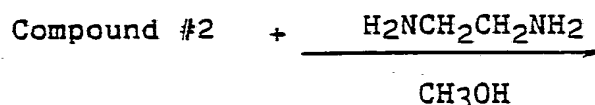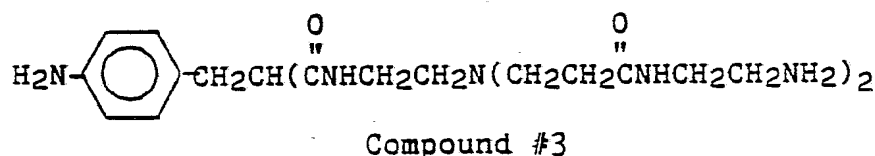g). No carboxyethylation of the aniline function was
observed.

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Example H: Preparation of a starburst polymer
(containing an aniline moiety) of one generation;
represented by the following scheme:

5   Compound #2   + $\underline{H_2NCH_2CH_2NH_2}$

                                  CH_3OH

10

$$H_2N\!-\!\!\bigcirc\!\!-CH_2CH(\overset{O}{\overset{\|}{C}}NHCH_2CH_2N(CH_2CH_2\overset{O}{\overset{\|}{C}}NHCH_2CH_2NH_2)_2$$
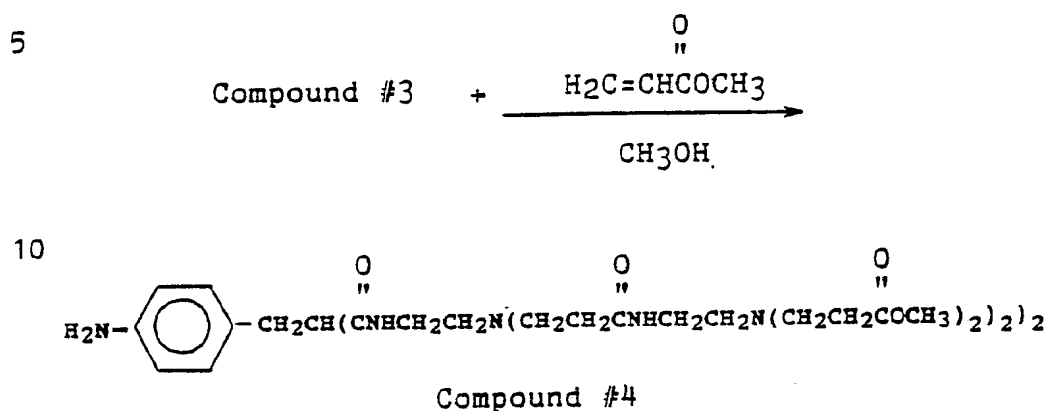
                  Compound #3

15

        The ester (Compound #2) (2.6 g, 3.7 mmole) was
dissolved in methanol (100 ml). this was carefully
added to a vigorously stirring solution of ethylene
diamine (250 g, 4.18 mole) and methanol (100 ml) at
20   such a rate that the temperature did not rise above
40°C. After complete addition the reaction mixture was
stirred for 28 hours at 35-40°C (heating mantle). After
28 hours no ester groups were detectable by infrared
spectroscopy. The solvent was removed on the rotary
25   evaporator at 60°C. The excess ethylene diamine was
removed using a ternary azeotrope of toluene-methanol-
ethylene diamine. Finally all remaining toluene was
azeotroped with methanol. Removal of all the methanol
30   yielded 3.01 g of the product (Compound #3) as an
orange glassy solid.

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Example I: Preparation of a starburst polymer (containing an aniline moiety) of one and one half generations represented by the following scheme:

$$
\text{Compound \#3} \quad + \quad \underset{CH_3OH}{\overset{\overset{\displaystyle O}{\overset{\|}{H_2C=CHCOCH_3}}}{\xrightarrow{\hspace{3cm}}}}
$$

$$
H_2N-\bigcirc-CH_2CH(\overset{\overset{\displaystyle O}{\|}}{C}NHCH_2CH_2N(CH_2CH_2\overset{\overset{\displaystyle O}{\|}}{C}NHCH_2CH_2N(CH_2CH_2\overset{\overset{\displaystyle O}{\|}}{C}OCH_3)_2)_2)_2
$$

Compound #4

The amine (Compound #3) (2.7 g, 3.6 mmole) was dissolved in methanol (7 ml) and was added slowly over one hour to a stirred solution of methyl acrylate (3.8 g, 44 mmole) in methanol (15 ml) at ambient temperatures. A slight warming of the solution was observed during the addition. The solution was allowed to stir at ambient temperatures for 16 hours. The solvent was removed on the rotary evaporator at 40°C. After removal of all the solvent and excess methyl acrylate the ester (Compound #4) was obtained in 4.7 g yield as an orange oil.
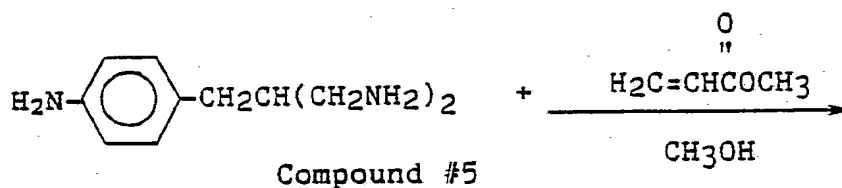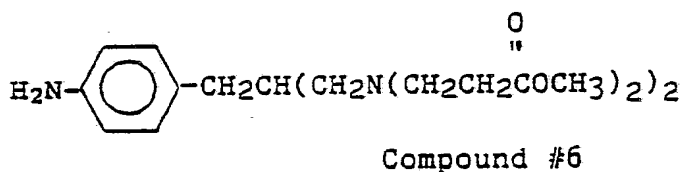
-58-

Example J: Preparation of a starburst polymer
(containing an aniline moiety) of one half generation
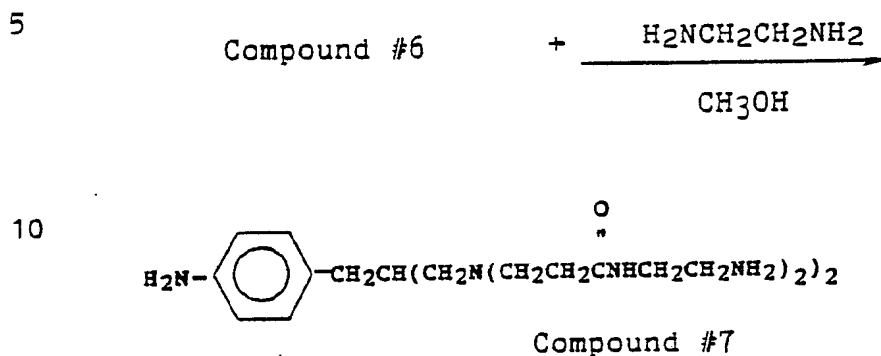represented by the following scheme:

5

$$H_2N-\langle\!\bigcirc\!\rangle-CH_2CH(CH_2NH_2)_2 \quad + \quad \overset{\overset{O}{\underset{\parallel}{}}}{\underset{CH_3OH}{\underline{H_2C=CHCOCH_3}}} \longrightarrow$$

Compound #5

10

$$H_2N-\langle\!\bigcirc\!\rangle-CH_2CH(CH_2N(CH_2CH_2\overset{\overset{O}{\underset{\parallel}{}}}{C}OCH_3)_2)_2$$

15   Compound #6

The triamine (Compound #5, the preparation of
20 this compound is shown in Example C) (0.42 g, 2.3
mmole) was dissolved in methanol (10 ml) and was added
dropwise over one hour to methyl acrylate (1.98 g, 23
mmole) in methanol (10 ml). The mixture was allowed to
stir at ambient temperatures for 48 hours. The solvent
25 was removed on the rotary evaporator, maintaining the
temperature at no higher than 40°C. The excess methyl
acrylate was removed by repeated azeotroping with
methanol. The ester (Compound #6) was isolated as an
30 orange oil (1.24 g).

35

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Example K: Preparation of a starburst polymer
(containing an aniline moiety) of one generation;
represented by the following scheme:

5

Compound #6   + $\underline{H_2NCH_2CH_2NH_2}$

                            CH_3OH

10

$H_2N-\langle\bigcirc\rangle-CH_2CH(CH_2N(CH_2CH_2\overset{O}{\overset{\|}{C}}NHCH_2CH_2NH_2)_2)_2$

Compound #7

15

     The ester (Compound #6) (1.24 g, 2.3 mmole) was
dissolved in methanol (50 ml) and was added dropwise
over two hours to ethylenediamine (73.4 g, 1.22 mole)
in methanol (100 ml). A small exotherm was noted,

20 vigorous stirring was maintained. The solution was
left to stir at ambient temperatures for 72 hours. The
solvent was removed on the rotary evaporator at 60°C.
The excess ethylene diamine was removed using a ternary
azeotrope of toluene-methanol-ethylenediamine. Finally

25 all remaining toluene was removed with methanol and
then pumping down with a vacuum pump for 48 hours gave
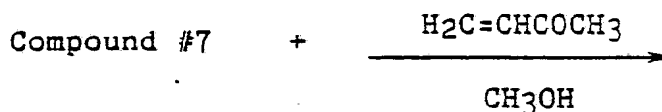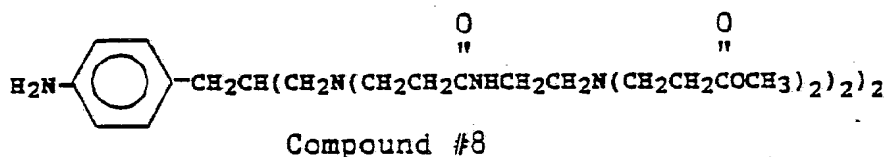the amine (Compound #7) (1.86 g) as a yellow/orange
oil.

30

35

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Example L: Preparation of a starburst polymer
(containing an aniline moiety) of one and one half
generations; represent by the following scheme:

5

$$Compound \ \#7 \quad + \quad \xrightarrow[CH_3OH]{H_2C=CHCOCH_3}$$

10

$$H_2N-\underset{}{\bigcirc}-CH_2CH(CH_2N(CH_2CH_2C\overset{O}{\overset{\|}{N}}HCH_2CH_2N(CH_2CH_2C\overset{O}{\overset{\|}{O}}CH_3)_2)_2)_2$$

Compound #8

15

The amine (Compound #7) (1.45 g, trace of
methanol remained) was dissolved in methanol (100 ml)
and was added slowly over 1½ hours to a stirred
solution of methyl acrylate (5.80 g) in methanol (20
ml). The solution was allowed to stir for 24 hours at
room temperature. Removal of the solvent followed by
repeated azeotroping with methanol enabled the removal
of all the excess methyl acrylate. After pumping down
on a vacuum pump for 48 hours the ester (Compound #8)
was isolated as an orange oil (2.50 g, 1.8 mmole).

Example M: Hydrolysis of 4.5 generation dendrimer and
preparation of calcium salt.

4.5 Generation PAMAM (ester terminated,
initiated off NH_3) (2.11 g, 10.92 meq) was dissolved in
25 ml of methanol and to it was added 10% NaOH (4.37
ml, 10.92 meq) (pH = 11.5-12). After 24 hours at room
temperature, the pH was about 9.5. After an additional

20 hours, the solution was rotovaped (rotary
evaporated), 50 ml of toluene added, and rotovaped
again.

5   The resulting oil was dissolved in 25 ml of
methanol and precipitated as a white gum upon addition
of 75 ml of diethyl ether. The liquid was decanted,
and the gum was rotovaped to give a very fine off-white
powder which upon further drying gives 2.16 g of
10   product (98% yield). No ester groups were found upon
NMR and infrared analysis.

        The sodium salt of 4.5 Generation PAMAM (ester
terminated, initiated from NH3) was replaced by the
15   calcium salt via dialysis. The sodium salt (1.03 g)
was dissolved in 100 ml of water and passed through
hollow fiber dialysis tubing (cut off = 5000) at 3
ml/minute. The exterior of the tubing was bathed in 5%
CaCl2 solution. This procedure was then repeated.
20
        The resulting solution was again dialyzed, this
time against water, then repeated two additional times.

        Evaporation provided 0.6 g of wet solid, which
25   was taken up in methanol (not totally soluble) and is
dried to give 0.45 g of off-white crystals.

        $C_{369}H_{592}O_{141}N_{91}Ca_{24}$ Calc. - 10.10% Ca^{++}

30   M Wt. = 9526.3 Calc. = C-4432.1, H-601.8, O-2255.9,
                                    N-1274.6, Ca-961.9)

        Theo: C-46.5, H-6.32, N-13.38, Ca-10.10
        Found: C-47.34, H-7.00, N-13.55, Ca-8.83

35

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Example N: Preparation of dendrimers with terminal
carboxylate groups.

Half-generation starburst polyamidoamines were
hydrolyzed to convert their terminal methyl ester
groups to carboxylates. This generated spheroidal
molecules with negative charges dispersed on the
periphery. The dendrimers hydrolyzed ranged from 0.5
generation (three carboxylates) to 6.5 generation (192
carboxylates).

The products could be generated as Na^+, K^+, Cs^+
or Rb^+ salts.

Example O: N-t-butoxycarbonyl-4-aminobenzyl malonate
dimethylester

4-Aminobenzyl malonate dimethylester (11.62 g,
49 mmol) was dissolved in 50 ml of t-butanol:water
(60:40 with stirring. Di-t-butoxydicarbonate (19.79 g,
90 mmol) was added and the reaction mixture stirred
overnight. The butanol was removed on the rotary
evaporator, resulting in a yellow suspension of the
product in water. Extraction into methylene chloride,
drying ($MgSO_4$) and evaporation gave a yellow oil (21.05
g, contaminated by di-t-butoxydicarbonate).
Recrystallization from 2-propanol:water (75:25) yield
pale yellow crystals (11.1 g, 33 mmol, 67%). The
structure was confirmed by ^{13}C NMR and purity checked
by hplc analysis (spherisorb ODS-1, 0.05M H_3PO_4 pH 3:
CH_3CN 55:45). The material was used without further
purification.

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Example P: N-t-butoxycarbonyl-6-(4-aminobenzyl)-
1,4,8,11-tetraaza-5,7-dioxoundecane

N-t-butoxycarbonyl-4-aminobenzyl malonate
dimethylester (8.82 g 26 mmol), prepared in Example O,
5   was dissolved in 50 ml of methanol, This solution was
added dropwise (2 hours) to a solution of freshly
distilled ethylenediamine (188 g 3.13 mole) and 20 ml
of methanol, under a nitrogen atmosphere. The solution
was allowed to stir for 24 hours. The ethylene
10   diamine/methanol solution was removed on the rotary
evaporator. The product was dissolved in methanol and
toluene added. Solvent removal on the rotary
evaporator gave the crude product as a white solid
(10.70 g contaminated with ethylenediamine). The
15   sample was divided into two samples for purification.
Azeotropic removal of ethylenediamine with toluene,
using a soxhlet extractor with sulphonated ion exchange
beads in the thimble to trap the ethylenediamine,
20   resulted in partial decomposition of the product,
giving a brown oil. The remaining product was isolated
as a white solid from the toluene on cooling (2.3 g
approximately 50 percent). Analysis of a 10 percent
solution in methanol by gas chromatography (Column,
25   Tenax 60/80) showed no ethylenediamine detectable in
the sample (<0.1 percent). The second fraction was
dissolved in methanol to give a 10 percent solution (by
weight) and purified from the ethylenediamine by
reverse osmosis, using methanol as the solvent. (The
30   membrane used was a Filmtec FT-30 , in an Amicon TC1R
thin channel separator, the ethylenediamine crossing
the membrane.) The product was isolated as a white
solid (2.7 g), in which no detectable amounts of
35   ethylenediamine could be found by gas chromatography.
The ^{13}C NMR data and HPLC analysis (Spherisorb ODS-1,

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0.05M H_3PO_4 pH 3:CH_3CN 55:45) were consistent with the
proposed structure. The product was used with no
further purification.

5   Example Q: Preparation of a starburst dendrimer of one
half generation from N-t-butoxycarbonyl-6-(4-
aminobenzyl)-1,4,8,11-tetraaza-5,7-dioxoundecane

        N-t-butoxycarbonyl-6-(4-aminobenzyl)-1,4,8,11-
tetraaza-5,7-dioxoundecane (5.0 g 13 mmol), prepared in
10 Example P, was dissolved in 100 ml of methanol. Methyl
acrylate (6.12 g, 68 mmol) was added and the solution
stirred at ambient temperatures for 72 hours. The
reaction was monitored by HPLC (Spherisorb ODS1,
Acetonitrile: 0.04M Ammonium acetate 40:60) to optimize
15 conversion to the desired product. The solution was
concentrated to 30 percent solids, and methyl acrylate
.(3.0 g 32 mmol) was added. The reaction mixture was
stirred at ambient temperatures until no partially
alkylated products were detectable by HPLC (24 hours).
20 Removal of the solvent at 30°C by rotary evaporation,
and pumping down at 1 mm Hg for 24 hours gave the
product as yellow viscous oil, yield 7.81 g. The 13C
NMR data was consistent with the proposed structure.
25 The product was used without further purification.

Example R: Preparation of a starburst dendrimer of one
full generation from N-t-butoxycarbonyl-6-(4-
aminobenzyl)-1,4,8,11-tetraaza-5,7-dioxoundecane
30
        The half generation product (Example Q) (7.70
g, 10.45 mmol) was dissolved in 75 ml of methanol and
added dropwise over 2 hours to a stirred solution of
ethylenediamine (400 ml, 7.41 mol) and methanol (50
35 ml). The reaction mixture was stirred at ambient
temperatures for 48 hours. The ethylenediamine and

methanol were removed by rotary evaporation to give a
yellow oil (11.8 g contaminated with ethylenediamine).
The product was dissolved in 90 ml of methanol, and
purified from the ethylenediamine by reverse osmosis

5   (Filmtec FT-30 membrane and Amicon TC1R thin channel
separator, methanol as solvent). After 48 hours, no
ethylenediamine could be detected by gas chromatography
(Column, Tenax 60/80). Removal of the solvent on the
rotary evaporator, followed by pumping down on a vacuum

10   line for 24 hours gave the product as a yellow glassy
solid (6.72 g). Analysis by HPLC, PLRP-S column,
acetonitrile:0.015M NaOH, 10-20 percent gradient in 20
min.) and 13C NMR analysis was consistent with the
proposed structure.

15

Example S: Preparation of a starburst polymer of one
and one half generation from N-t-butoxycarbonyl-6-(4-
aminobenzyl)-1,4,8,11-tetraaza-5,7-dioxoundecane

20   The one generation product (Example R) (2.14 g,
25 mmol) was dissolved in 12.5 ml of methanol, and
methyl acrylate (3.5 g, 39 mmol) in 5 ml of methanol
was added. The solution was stirred at ambient
temperatures for 48 hours, monitoring the progress of

25   the reaction by HPLC (Spherisorb ODS-1, acetonitrile:
0.04M ammonium acetate, 60:40). A second aliquot of
methyl acrylate was added (3.5 g 39 mmol) and the
reaction mixture stirred at ambient temperatures for a
further 72 hours. Removal of the solvent on the rotary

30   evaporator gave the product as a yellow oil (3.9 g)
after pumping down overnight with a vacuum pump. The
product was used with no further purification.

35

Example T: Preparation of a starburst polymer of two
full generations from N-t-butoxycarbonyl-6-(4-
aminobenzyl)-1,4,8,11-tetraaza-5,7-dioxoundecane

    The one and one half generation product
5   (Example S) (3.9 g, 2.5 mmol) was dissolved in 50 ml of
methanol, and was added dropwise over 2 hours to a
stirred solution of ethylenediamine (600 g, 10 mol) and
methanol (50 ml). The solution was stirred at ambient
temperatures under an atmosphere of nitrogen for 96
10   hours. The ethylenediamine/methanol was removed on the
rotary evaporator to give a yellow glassy solid (4.4 g
contaminated with ethylenediamine). A 10 percent
solution of the product was made in methanol, and
purified from the ethylenediamine by reverse osmosis
15   (membrane used as a Filmtec FT-30, in an Amicon TC1R
thin channel separator) until no ethylenediamine could
be detected by gas chromatography (Column, Tenax 60/80.
Removal of the solvent gave the product as a yellow
glassy solid (3.52 g). The 13C NMR data and HPLC
20   analysis (PLRP-S column, acetonitrile:0.015 M NaOH, 10
to 20 percent gradient in 20 minutes, were consistent
with the proposed structure.

25   Example U: Reaction of the two generation starburst
with Bromoacetic Acid to give a methylene carboxylate
terminated starburst dendrimer

    The second generation product (Example T) (0.22
g, 0.13 mmol) was dissolved in 15 ml of deionized water
30   and the temperature equilibrated at 40.5°C. Bromoacetic
acid (0.48 g, 3.5 mmol) and lithium hydroxide (0.13 g,
3.3 mmol) were dissolved in 5 ml of deionized water,
and added to the reaction mixture. The reaction pH was
35   carefully maintained at 9, with the use of a pH stat
(titrating with 0.1N NaOH), at 40.5°C overnight.

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Monitoring by reverse phase HPLC, (Spherisorb ODS-1
column, eluent 0.25 M H_3PO_4 pH 3 [NaOH]; acetonitrile
85:15) confirmed the synthesis of predominantly a
single component.

5

Example V: Preparation of Isothiocyanato functionalized
second generation methylene-carboxylate terminated
starburst dendrimer

    Five ml of a 2.8 mM solution of the second
10 generation methylenecarboxylate terminated starburst
dendrimer (Example U) was diluted with 20 ml water and
the pH adjusted to 0.5 with concentrated hydrochloric
acid. After one hour at room temperature the mixture
was analyzed by HPLC to verify the removal of the
15 butoxycarbonyl group and then treated with 50 percent
sodium hydroxide to being the pH to 7. A pH stat
(titrating with 0.1 N NaOH) was used to maintain the pH
at 7 and 225 µl thiophosgene was added. After 15
20 minutes at room temperature the pH of the mixture was
adjusted to 5 with 1N HCl. The mixture washed with
chloroform (20 ml x 2) then concentrated on a rotary
evaporator at reduced pressure. The residue recovered
0.91 g is a mixture of the isothiocyanate and salts.
25

Example W: Preparation of second generation starburst
polyethyleneimine-methane sulfonamide

    To a solution of 125 g N-methanesulfonyl-
30 aziridine in 50 ml ethanol was added 25.0 g tris(2-
aminoethyl)amine. The solution was stirred at room
temperature for 4 days. Water was added to the
reaction mixture as needed to maintain the homogeneity
of the solution. The solvent was removed by
35 distillation in vacuo to give the 2nd generation

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starburst PEI-methane sulfonamide as a yellow glass
(161 g).

Example X: Cleavage of methane sulfonamides to form
second generation starburst polyethyleneimine

A solution of 5.0 g of second generation
starburst PEI-methane sulfonamide, from Example W in 20
ml of 38 percent HCL was sealed in a glass ampoule.
The ampoule was heated at 160°C for 16 hours, then
cooled in an ice bath and opened. The solvent was
removed by distillation in vacuo and the residue
dissolved in water. After adjusting the pH of the
solution to greater than or equal to 10 with 50 percent
NaOH, the solvent was removed by distillation in vacuo.
Toluene (150 ml) was added to the residue and the
mixture heated at reflux under a Dean-Stark trap until
no more water could be removed. The solution was
filtered to remove salts and the filtrate concentrated
in vacuo to give 1.9 g second generation starburst PEI
as a yellow oil.

Example Y Preparation of third generation starburst
polyethyleneimine-methane sulfonamide

To a solution of 10.1 g second generation
starburst PEI, from Example X, in 100 ml ethanol was
added 36.6 g N-methanesulfonylaziridine. The solution
was stirred at room temperature for 1 week. Water was
added as needed to maintain the homogeneity of the
solution. The solvent was removed by distillation in
vacuo to give third generation starburst PEI-methane
sulfonamide as a yellow glass (45.3 g).

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Example Z: Cleavage of methane sulfonamides to form 3rd generation starburst polyethyleneimine

5   The methane sulfonamide groups of third generation starburst PEI-methane sulfonamide (5.0 g), from Example Y, were removed by the same procedure as described for the second generation material in Example X to give 2.3 g third generation starburst PEI as a
10   yellow oil.

Example AA: Reaction of a third generation starburst polyethyleneimine with 4-fluoro-nitrobenzene

       The third generation starburst
15   polyethyleneimine (Example Z) (1.06 g, 1.2 mmol) was dissolved in 12 ml of absolute ethanol. (4-Fluoro)-nitrobenzene (120 µl, 1.2 mmol) was added and the reaction mixture refluxed overnight. The solvent was removed on the rotary evaporator, and the bright yellow
20   oil dissolved in water. The aqueous solution was washed with chloroform to remove any unreacted (4-fluoro)-nitrobenzene. Removal of the water gave the product as a deep yellow oil (0.80 g). The ^{13}C NMR
25   spectrum was consistent with the proposed structure. (No attempt was made to determine the nature of the statistical distribution). The product was used without further purification.

30   Example BB: Reaction of the nitrophenyl derivative of the third generation starburst polyethyleneimine with glycolonitrile.

       The nitrophenyl derivative of the third
35   generation starburst polyethyleneimine (Example AA) (0.80 g) was dissolved in 20 ml of deionized water.

Sodium hydroxide (2.80 g, 50 percent w/w) was added to
the stirred solution, and the solution purged with
nitrogen, venting through a sodium hydroxide scrubber.
Glycolonitrile (2.85 ml of a 70 percent aqueous

5   solution) was added at ambient temperatures. A yellow
precipitate was observed to form after a few minutes.
After two hours, the temperature was slowly raised to a
reflux, and the solution maintained at a reflux with a
nitrogen purge for 24 hours. Removal of the water gave

10   the product as a yellow solid contaminated with
glycolic acid and sodium hydroxide. The ^{13}C NMR
spectrum was consistent with the proposed structure.
The product was used without further purification.

15
Example CC: Hydrogenation of the nitrophenyl derivative
to the aminophenyl methylenecarboxylate terminated
third generation starburst polyethyleneimine.

       The yellow solid from Example BB (1.70 g) was

20   dissolved in 10 ml of deionized water, the resulting pH
of the solution was approximately 11. Palladium on
charcoal (200 mg of 5 percent Pd/C) was added to the
reaction mixture in a glass Parr shaker bottle. The
reaction mixture was placed under a pressure of 40 psi

25   (275 kPa) of hydrogen, and shaken at ambient
temperature in a Parr hydrogenation apparatus, for 6
hours. The reaction mixture was then filtered through
a 0.5 m Millipore filter to remove the Pd/C and the

30   solvent removed in vacuo and was gel filtered through a
Biogel P2 resin (25 g swollen with water).
Acidification with HCl resulted in an orange brown
solution, which was purged with nitrogen overnight.
Removal of the solvent in vacuo gave the product as the

35   hydrochloride salt which was a pale brown solid (3.98
g, contaminated with NaCl and glycolic acid, maximum

theoretical amount of product 1.15g). The product was
used with no further purificate.

Example DD: Preparation of 4-isothiocyanatophenyl
methylenecarboxylate terminated third generation
starburst polyethyleneimine

          The product from Example CC (3.98 g) was
dissolved in 15 ml of deionized water and an aliquot
(2.5 ml) of this solution was diluted with 10 ml water.
the pH of the solution was adjusted to 7 with sodium
hydroxide. A pH stat (titrating with 1N NaOH) was used
to maintain the pH and 200 µl thiophosgene was added.
After 10 minutes the pH of the mixture was adjusted to
4 with hydrochloric acid. Water was removed on a
rotary evaporator at reduced pressure (a small amount
of n-butanol was added to prevent foaming) . The
residue was washed with methylene chloride and then
dried. The crude product (0.95 g) a mixture of
isothiocyanate (0.14 g) and salts was used without
further purification.

Example EE: Preparation of a methylenecarboxylate-
terminated second generation starburst polyamidoamine
(initiated from ammonia)

          The second generation starburst polyamidoamine
(2.71 g, 2.6 mmol) and bromoacetic acid (4.39 g, 31.6
mmol) were dissolved in 30 ml of deionized water and
the pH adjusted to 9.7 with 5N NaOH using a pH stat.
The reaction was maintained at this pH for a half hour,
and the temperature was slowly raised to 60°C and was
maintained at 60°C for three hours at constant pH. The
pH was raised to 10.3, and the reaction mixture

remained under control of the pH stat at ambient
temperatures overnight. The reaction mixture was
refluxed for a further four hours prior to work up.
Removal of the solvent, and azeotroping the final
5   traces of water with methanol gave the product as a
pale yellow powder (8.7 g, contaminated with sodium
bromide). The ^{13}C NMR spectrum was consistent with the
propose structure (with some contamination due to a
small amount of defected material as a result of some
10   monoalkylation).

Example FF: Preparation of a methylenecarboxylate
terminated second generation starburst
polyethyleneimine (initiated from ammonia)

15
       The second generation starburst
polyethyleneimine (2.73 g, 6.7 mmol), from Example X,
and bromoacetic acid (11.29 g 81 mmol) were dissolved
in 30 ml of deionized water. The pH was slowly raised
20   to pH 9.5 maintaining the temperature below 30°C. The
temperature was raised slowly to 55°C, and the reaction
pH maintained at 9.5 for 6 hours with the aid of a pH
stat (titrating with 5N NaOH). The pH was raised to
10.2, and maintained at that pH overnight. Removal of
25   the solvent on the rotary evaporator, and azeotroping
the final traces of water using methanol, gave the
product as a yellow powder (17.9 g, contaminated with
sodium bromide). The ^{13}C NMR spectrum was consistent
with the proposed structure (with some contamination
30   due to a small amount of defected material as a result
of some monoalkylation).

35

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Example GG: Preparation of a 3.5, 4.5, 5.5 and 6.5
generation starburst PAMAM

     To a 10 wt% methanolic solution of 2.46 g 3
generation PAMAM starburst was added 2.32 g of methyl

5   acrylate. This mixture was allowed to sit at room
temeprature for 64 hr. After solvent and excess methyl
acrylate removal, 4.82 g of product was recovered (105%
of theoretical).

10
Preparation of higher 1/2 generation starburst PAMAM'S:

     Generations 4.5, 5.5 and 6.5 were prepared as
described above with no significant differences in

15 reactant concentrations, reactant mole ratios or
reaction times.

Example HH: Preparation of a 4, 5 and 6 generation
starburst PAMAM:

20
     To 2000 g of predistilled ethylenediamine was
added 5.4 g of 4 1/2 generation starburst PAMAM as a 15
wt% solution in methanol. This was allowed to sit at
room temperature for 48 hrs. The methanol and most of

25 the excess ethylenediamine were removed by rotary
evaporation under water aspirator vacuum at temperature
less than 60°C. The total wt of product recovered was
8.07 g. Gas chromatography indicated that the product
still contained 34 wt% ethylenediamine at this point.

30 A 5.94 g portion of this product was dissolved in 100
ml methanol and ultrafiltered to remove the residual
ethylenediamine. The filtration was run using an Amicon
TC1R thin channel recirculating separator equipped with

35 an Amicon YM2 membrane. An in-line pressure relief
valve was used to maintain 55 psig (380 kPa) pressure

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across the membrane. The 100 ml was first concentrated
to 15 ml by forcing solvent flow exclusively through
the membrane. After this initial concentration, the
flow was converted to a constant volume retentate
5 recycle mode for 18 hrs. After this time, 60 ml of
methanol was passed over the membrane to recover
product still in the module and associated tubing. The
product was stripped of solvent and 2.53 g of 5
generation starburst PAMAM was recovered. Analysis by
10 gas chromatography indicated 0.3% residual
ethylenediamine remained in the product.

        Preparation of generation 4 and 6 proceeded as
15 above with the only difference being the weight ratio
of ethylenediamine to starting material. To prepare
4th generation this ratio was 200:1 and for 6th
generation this ratio was 730:1.

20
Example 1: Incorporation of 2-(acetyloxy)benzoic acid
(aspirin) into starburst dendrimers.

        A widely accepted method for ascertaining
25 whether a "probe molecule" is included in the interior
of a micelle is to compare its carbon-13-spin lattice
relaxation times (T_1) in a non-micellized versus
micellized medium. A substantial decrease in T_1 for
the micellized medium is indicative of "probe molecule"
30 inclusion in the micelle. Since starburst dendrimers
are "covalently fixed" analogs of micelles, this T_1
relaxation time technique was used to ascertain the
degree/extent to which various pharmaceutical type
molecules were associated with starburst
35 polyamidoamines. In the following examples, T_1 values
for (acetyloxy)benzoic acid (I) (aspirin) were

determined in solvent (CDCl3) and then compared to T_1
values in CDCl3 at various [I:dendrimer] molar ratios.

     Inclusion of aspirin (I) into various starburst
polyamidoamine dendrimers as a function of generation.

     Various half generation (ester terminated,
initiated from NH3) starburst polyamidoamine dendrimers
(G = 0.5 → 5.5) were combined with 2-(acetyloxy)benzoic
acid in CDCl3 to give acid:tertiary amine ratios of =
1.0. A plot of T_1 values for 2-(acetyloxy)benzoic acid
versus generation of starburst dendrimer added (see
Figure 4 where ·represent C-4, п represent C-6, and o
represent C-5) shows that T_1 reaches a minimum over the
generation range of 2.5 → 5.5 for carbons 4, 5 and 6 in
2-(acetyloxy)benzoic acid. This demonstrates
association of 2-(acetyloxy)benzoic acid in the
dendrimers (G = 2.5 → 5.5) and further confirms that
polyamidoamine dendrimers (Gen = 2.5 or greater) can
function as carrier molecules.

Example 2 Release of pseudoephedrine from starburst
dendrimer - PAMAM

     Pseudoephedrine (0.83 mg/ml) and starburst
PAMAM dendrimer [1.0 mg/ml; G= 6.5; terminal group (Z) =
192 (methyl ester)] were dissolved in deionized
distilled water and the pH of the donor phase was
adjusted to 9.5, with sodium hydroxide solution, and
stored at room temperature for about 12 hours.
Solution of pseudoephedrine alone was treated in the
same way (control). The drug dendrimer solution was
stored at 40°C for 8 hours after the first experiment
and dynamic dialysis performed. Dialysis membrane used
was a spectra/Por 7, MWCO 1,000 28.6 mm in diameter in

spectrum separation cells (half cell volume 5 and 10 ml, cell dimensions: 38 mm diameter for both the cells and the cell depth of 10 and 20 mm for 5 and 10 ml cells, respectively).

5

Samples were analyzed by a HPLC procedure developed for pseudoephrine conditions for which are as follows:

10

       Column: uBondapak C-18
       Mobile phase: pH 3.2 phosophate buffer plus
                       acetonitrile (80:20)
       Flow rate : 0.3 ml/min

15
       Detection: UV at 210 nm
       Retention time: 13.3 min

The dialysis membrane was washed with deionized
20 water and was kept soaking in the receptor phase for at least 12 hours prior to use. The dialysis membrane was placed in between the donor and the receptor compartment was stirred with a small magnetic spin bar. Known volumes of donor and receptor solutions were
25 introduced into the respective compartments and transfer of pseudoephedrine to the receptor compartment was followed as a function of time. To maintain sink conditions the entire receptor phase was removed
30 periodically (every 30 minutes) and replaced with fresh receptor phase. The amount of pseudoephedrine was assayed in the sampled receptor phase. Experiments were conducted at room temperature (22°C). The receptor phase was plain deionized distilled water.

35

The results of dynamic analysis are shown in
Figure 5. In Figure 5, the •represents pseudoephedrine
only (control), the   represents pseudoephedrine plus
the dendrimer, and the O represents pseudoephedrine

5   plus the dendrimer at 40°C, 8 hours before dialysis. It
is apparent that in presence of G = 6.5 dendrimer in
the donor compartment the rate of dialysis of
pseudoephedrine is reduced. Storing the donor solution
at 40°C, appears to further reduce the rate of dialysis.

10

The experiment was repeated at lower
concentrations (the ratio of number of drug molecules
to the number of terminal groups was kept the same).

15   G = 6.5 dendrimer 120 μ/ml pseudoephedrine 100 μ/ml
(122 μ/ml salt).

Dynamic dialysis of pseudoephedrine (alone) at
this lower concentration was almost identical to that

20   at higher concentration. Figure 6 summarizes the
results of this experiment where • represents
pseudoephedrine only (control), and o represents
pseudoephedrine plus dendrimer.

25

Example 3

The procedure of Example 2 was repeated using
the modifications given below.

Receptor phase: pH 7.4 phosphate buffer

30

Donor phase: pH 7.4 phosphate buffer plus
drug and dendrimer in the following ratios:

35

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1.   G 6.5 : Drug   ::   1 : 192

5   2.   G 5.5 : Drug   ::   1 : 96

3.   G 4.5 : Drug   ::   1 : 48

4.   G 6.5H: Drug   ::   1 : 192

5.   G 5.5H: Drug   ::   1 : 96

6.   G 4.5H: Drug   ::   1 : 48

10

The above donor phase compositions plus
pseudoephedrine alone were subjected to dynamic
dialysis. The letter "H" after the dendrimer
15 generation number stands for hydrolyzed dendrimer..
Hydrolysis was accomplished by the procedure described
in Examples M and N.

The results of these experiments are summarized
20 in Figure 7 where the donor and receptor compartment
contained pH 7.4 phosphate buffer. For pseduoephedrine
alone (P) the mean curve of three experiments is
plotted (shown by the solid line), and one typical run
25 from the other experiments are shown. In Figure 7, the
following symbols represent the dendrimer of the
indicated generation.

30

35

Table III

Symbol	Dendrimer Generation
O	5.5
●	6.5
O	4.5
⊖	5.5H
O	6.5H
O	4.5H

Pseudoephedrine appears not to associate with the dendrimer (unhydrolized) at pH 7.4. Hydrolysis of the terminal functional groups into carboxylate form, has a dramatic effect on the dialysis rate (reduction). The generation number appears not to influence the dialysis rate.

Example 4: Interaction studies of salicylic acid with PAMAM starburst dendrimers

This example evaluated interaction characteristics of salicyclic acid with PAMAM starburst dendrimers. These dendrimers consisted of an ammonia initiated core with repeating units derived from N-(2-aminoethyl) acrylamide. Both full (amine terminated functions) and half (ester terminal groups) generation polymers were included in the studies. The ratio of salicyclic acid to starburst dendrimers utilized in the experiments resulted in approximately one salicyclic acid molecule to one terminal amine functional group for full generation polymers. In the half-generation

polymer study, the same ratio was employed with
adjustments made for the higher molecular weight
polymer.

5   The experiments were conducted at room
temperature using an equilibrium static cell dialysis
methadology. Spectrum static dialysis cells (half cell
volume, 10 ml) separated by SpectraPor 6 membranes
(molecular weight cutoff = 1000) were utilized for all
10 experiments. Transport of salicyclic acid was
monitored as a function of time by removing aliquots
from appropriate cell compartments and assayed by HPLC
analysis using a U.V. detector at 296 nm (Bondupak C-
18 Column, eluting mobile phase of acetonitrile/0.1M
15 phosphate buffer (pH 3.2) at a ratio of 20:80 (V/V),
set at a flow rate of 30 ml/hour).

       Ten ml of a solution containing 1 mg/ml
salicyclic acid and 2.5 mg/ml starburst polymer (Gen
20 4.0) adjusted to pH 6.65 and 5.0 with HCL solution were
placed in the donor compartment of the dialysis cell
and an equal volume of purified water adjusted to the
same pH's placed in the receptor compartment.
Transport of salicyclic acid into the receptor
25 compartment was monitored. The results are given in
Figure 8. In Figure 8, the free acid is represented by
●, the acid plus generation 4.0 dendrimer, pH 6.65 is
represented by 0, and the acid plus generation 4.0
dendrimer, pH 5.00 is represented by □.
30

       Due to the lower percent ionization of the
amine groups on the polymer at pH 6, a greater extend
of interaction with salicylic may be expected at pH 5,
35 resulting in less compound transported at the lower pH.
The results given in Figure 8 indicate a much lower

-81-

percentage of salicylic acid transported in the
presence of polymer at both pH's studied compared to
the salicyclic acid control study. It is also observed
that more salicyclic acid is transported at pH 6.65
5   than at pH 5.0 as predicted. The data demonstrates an
interaction of the starburst polymer with salicylic
acid that can be controlled by pH. Sustained release
characteristics are also implied by the data since the
salicyclic acid levels in the presence of polymer
10   continue to rise past the approximate 12-hour
equilibrium point observed in the control study.

     To further investigate the interaction
characteristics of salicylic acid with starburst
15   polymers (Gen = 4.0) an experiment was designed at pH
8.0. The design of the study differed from that
previously described in that only the salicylic acid
solution (1 mg/ml), adjusted to pH 8.0, was placed in
the donor compartment and the polymer solution (2.5
20   mg/ml) placed in the receptor compartment. Loss of
salicylic acid from the donor compartment was monitored
as previously described. The results of the experiment
are given in Figure 9. In Fig 9, the free acid is
25   represented by --•-- , and the acid plus generation 4.0
dendrimer at pH 8.0 is represented by --Δ----.

     As indicated in Figure 9, the equilibrium
characteristics of salicylic acid in the donor
30   compartment with starburst polymers in the receptor
compartment differs from the salicylic acid control
study. Based on the ionization characteristics of the
molecules at pH 8, approximately 6-7% interaction is
35   expected. The observed extent of interaction is
indicated to be on the order of 4-5%. The lower

-82-

association observed may be due to experimental
variability or to an ionization constant of less than
one.

5   This experiment indicates an uptake or removal
of free salicylic acid from the continuous phase of the
system by the polymer. This type of action could
result in suppression of reactivity of molecules
suggesting a possible chelating type of property
10   associated with the polymers.

The interaction characteristics of salicylic
acid at pH 6.65 with a half generation starburst
polymer (Gen = 4.5) having ester terminated functional
15   groups were evaluated. Salicylic acid (1 mg/ml) was
combined with starburst polymer (Gen = 4.5) 3.6 mg/ml
at pH 6.65. Ten ml of the solution was placed in the
donor compartment and transport from the donor
compartment was monitored as previously described. The
20   results are given in Figure 10. In Figure 10, the free
acid is represented by -●-, and the acid plus polymer
is represented by ---o---.

Under these experimental conditions no charge
25   interaction is predicted to occur since the tertiary
amine groups are non-ionized at pH 6.65. As is
indicated in Figure 10, the loss of salicylic acid in
the presence of polymer (Gen = 4.5) is virtually
30   identical during the first 10 hours of dialysis to that
of the salicylic acid control study.

The following observations are made from the
data presented in this example:

35

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    (1)   Full generation PAMAM starburst polymers
          function as a carrier for salicylic acid.

    (2)   Full generation PAMAM starburst polymers
5   have sustained release functionality for
          salicylic acid.

    (3)   Salicylic acid carrier properties of full
          generation PAMAM starburst polymers can be
          controlled by pH.

10

Example 5: Demonstration of multiple chelation of iron
by a sodium propionate terminated sixth generation
starburst polyamidoamine.

       The sodium propionate terminated sixth
15 generation polyamidoamine (initiated from ammonia)
(97.1 mg, 2.45 mol.) was dissolved in 1.5 ml of
deionized water. Addition of 0.5 ml of 0.5N HCl
reduced the pH to 6.3. Ferric chloride was added (0.5
ml of 0.1.2M solution, 0.051 mmol) producing a light
20 brown gelatinous precipitate. On heating at 60°C for
0.5 hours, the gelatinous precipitate became soluble,
resulting in a homogeneous orange solution. The
solution was filtered through Biogel P2 acrylamide gel
25 (10 g, twice) isolating the orange band (free of halide
contamination). Removal of the solvent in vacuo gave
the product as an orange film (30 mg). Analysis was
consistent with chelation of approximately 20 moles of
ferric ions per mole of starburst dendrimer.

30

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Table IV

Found	Theoretical		
	$Na_4Fe_{20}H_{128}SB$	$Na_5Fe_{20}H_{127}SB$	$Na_6Fe_{20}H_{126}SB$
Na 0.39,0.24 (0.31 0.1%)	0.25	0.31	0.38
Fe 3.14,3.11 (3.12 0.02%)	3.05	3.05	3.04
C 47.11	49.87	49.84	49.81
H 7.33	7.31	7.30	7.29
N 14.81	14.49	14.48	14.47
O ----	25.03	25.02	25.01
Mwt.	36632.23	36654.21	36375.18

$SB = C_{1521}H_{2467}N_{379}O_{573}$

These results confirm chelation of 20 ± 2 moles of ferric ions per mole of starburst dendrimer.

Example 6: Preparation of a product containing more than one rhodium atom per starburst polymer.

2.5 Gen PAMAM (ester terminated, initiated from NH_3) (0.18 g, 0.087 mmole) and $RhCl_3\cdot3H_2O$ (0.09 g, 0.3 mmole) were mixed in dimethylformamide (DMF) (15 ml) and heated for 4 hours at 70°C. The solution turned crimson and most of the rhodium was taken up. The unreacted rhodium was removed by filtration and the solvent removed on the rotary evaporator. The oil formed was chloroform soluble. This was washed with water and dried ($MgSO_4$) before removal of solvent to yield a red oil (0.18 g). The NMR spectrum was recorded in $CDCl_3$ only minor differences were noted between the chelated and unchelated starburst.

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Dilution of some of this CDCl3 solution with ethanol
followed by NaBH4 addition resulted in rhodium
precipitation. RhCl3•3H2O is insoluble in chloroform
and in chloroform starburst solution thus confirming
5   chelation.

Example 7: Preparation of a product containing Pd
chelated to a starburst polymer

10   3.5 Gen PAMAM (ester terminated, initiated from
NH3) (1.1 g, 0.24 mmole) was dissolved with stirring
into acetonitrile (50 ml). Palladium chloride (0.24 g,
1.4 mmole) was added and the solution was heated at 70-
75°C (water bath) overnight. The PdCl2 was taken up
15   into the starburst. After removal of the solvent, the
NMR in CDCl3 confirmed that chelation had occurred.
Dilution of the CDCl3 solution with ethanol and
addition of NaBH4 resulted in precipitation of the
palladium. The chelated product (1.23 g) was isolated
20   as a brown oil.

Example 8: Demonstration of multiple chelation of
yttrium by a methylene carboxylate terminated second
generation starburst polyethyleneimine by trans
25   chelation from yttrium acetate

       The starburst polyethyleneimine methylene
carboxylate terminated material (0.46 g 52. 5 percent
active, remainder sodium bromide, 0.18 mmol active
starburst dendrimer), from Example FF, was dissolved in
30   4.5 ml of deuterium oxide. The resultant pH was 11.5-
12. A solution of yttrium acetate was prepared by
dissolving yttrium chloride (0.15 g, 0.5 mmol) and
sodium acetate (0.41 g, 0.5 mmol) in 1.5 ml of
35   deuterium oxide (2.9 moles of yttrium per mole of
dendrimer). Aliquots of 0.5 ml of the yttrium acetate

solution were added to the dendrimer solution and the
^{13}C NMR spectra recorded at 75.5 MHz.

The ^{13}C NMR spectrum of yttrium acetate shows
two resonances, 184.7 ppm for the carboxyl carbon and
23.7 ppm for the methyl carbon, compared with 182.1 and
24.1 ppm for sodium acetate, and 177.7 and 20.7 ppm for
acetic acid (Sadtler ^{13}C NMR Standard Spectra).
Monitoring the positions of these bands indicates
degree of chelation with the starburst dendrimer. The
most informative signal for the starburst dendrimer
which is indicative of chelation is the α-CH_2 (of the
methylene carboxylate group involved in chelation),
which appears at 58.4 ppm in the unchelated dendrimer,
and 63.8 ppm in the chelated dendrimer. Upon chelation
with yttrium, the spin lattice relaxation times of the
time α-CH_2 shortens as expected from $0.24 \pm 0.01s$ to
$0.14 \pm 0.01s$, indicative of chelation.

Following the addition of 0.5 ml of the yttrium
acetate solution to the starburst dendrimer, all the
yttrium appeared to be chelated by the dendrimer,
confirmed by the signals for the acetate being that of
sodium acetate. The same observation was noted for the
addition of a second 0.5 ml aliquot of the yttrium
acetate solution. Upon addition of the third aliquot
of yttrium acetate, not all of the yttrium was observed
to be taken up as the starburst chelate, the acetate
carboxyl resonance was observed to shift to 183.8 ppm
indicating that some of the yttrium was associated with
the acetate. The integrated area of the chelated -CH_2
groups on the dendrimer increased, indicating that some
of the third mole equivalent of yttrium added was

· -87-

indeed chelated with the dendrimer. These results
indicate that the dendrimer can chelate from 2-3
yttrium ions per dendrimer molecule.

5   Example 9: Demonstration of Multiple Chelation of
Yttrium by a methylene carboxylate terminated second
generation starburst polyamidoamine by trans chelation
from yttrium acetate.

The same experimental methods were used for
10 this study as were used for Example 8. The starburst
polyamidoamine methylene-carboxylate terminated
material (0.40g 62.5% active, remainder sodium bromide,
0.12 mmol.) was dissolved in 4-5 ml of deuterium oxide.
The resultant pH was 11.5-12, which was lowered to 9.4
15 with 6N HCl prior to the experiment. A solution of
yttrium acetate was prepared by dissolving yttrium
chloride (0.1125g, .37 mmol.) and sodium acetate
(0.0915g, 1.1 mmol.) in 1.5 ml of deuterium oxide, thus
every 0.5 ml of solution contains one mole equivalent
20 of metal.

The first two mole equivalents of yttrium
acetate added were fully chelated by the starburst
polyamidoamine. On addition of a third mole equivalent
25 of yttrium, precipitation of the product occurred and
as such no NMR data could be obtained. The signals
which gave the most information about chelation by the
starburst dendrimer were those of the two carbons
30 adjacent to the chelating nitrogen. The chemical
shifts of these carbons in the unchelated dendrimer
occurred at 59.1 ppm for the α-CH_2, and 53.7 ppm for
the first methylene carbon of the backbone. Upon
chelation these two resonances were observed to shift
35 downfield to 60.8 and 55.1 ppm respectively. The trans
chelation shows that two metal ions can be readily

chelated per dendrimer molecule, however upon chelation
of some unknown fraction of a third mole equivalent,
the product precipitates out of solution.

5 Example 10: Demonstration of Multiple Chelation of ^{90}Y
by a methylenecarboxylate terminated second generation
starburst polyethyleneimine.

Standard solution of yttrium chloride (3×10^{-2}
M, spiked with non-carrier added ^{90}Y) and
10 methylenecarboxylate terminated second generation
starburst polyethyleneimine (6×10^{-2} M) were prepared.
These were reacted together at various metal:starburst
ratios in HEPES buffer. The complex yield was
determined by ion exchange chromatography using
15 Sephadex G50 ion exchange beads, eluting with 10%
NaCl:NH$_4$OH, 4:1 at pH 10. Noncomplexed metal is
removed on the column, complexed metal elutes. Yields
were obtained by comparing the radioactivity eluted
20 with that on the column, using a well counter.

25

30

35

Table V

Chelation of 2.5 Gen. PEI Acetate with ^{90}Y

	Vol. Y+3	Vol. PEI	Vol HEPES	M:L Theor.	% Complex	M:L Act.
5	5	30	370	0.1	110	0.1
	10	30	360	0.2	101	0.2
	20	30	350	0.4	95	0.4
	30	35	340	0.5	97	0.5
10	30	30	340	0.5	102	0.5
	60	30	310	1.0	99	1.0
	120	30	250	2.0	100	2.0
	180	30	180	3.0	94	2.8
15	250	30	120	4.1	80	3.3
	300	20	80	7.5	44	3.3
	300	20	70	5.0	40	2.0
	300	20	70	5.0	41	2.0

20 All volumes in Table V are in microlitres

Within the accuracy of the experiments, these results
indicate that the 2.5 Gen. starburst PEI acetate can
chelate between 2 and 3 metals per polymer giving a
25 soluble complex.

Example 11: Conjugation of 4-isothiocyanatophenyl
methylenecarboxylate terminated third generation
starburst polyethyleneimine with IgG monoclonal
antibody
30
        The isothiocyanate, 10 mg (50 μ moles), from
Example DD was dissolved in 500 μl of 3 mM indium
chloride which had been spiked with radioactive indium-
111 chloride and the pH was adjusted to 9 with 660 μl N
35 NaOH. Aliquots of whole antibody IgG CC-46 was then
mixed with aliquots of the chelated starburst. The

mixtures were shaken then left for 18 hours. The
mixtures were then analyzed by HPLC (column Dupont
Zorbax Biosphere GF-250; eluent 0.25 M sodium acetate,
pH 6) and a UV detector at 254 nm and a radioactivty
5 detector. Results are shown in Table VI.

Table VI
Starburst-IgG Conjugates

	1	2	3	4
IgG solution (µl)	20	20	20	20
Chelated Starburst solution (µl)	5	20	50	100
% Radioactivity on IgG	6	5	5	3
% IgG conjugated	2	7	17	22

Example 12: Conjugation of 4-isothiocyanatophenyl
methylenecarboxylate terminated third generation
starburst polyethyleneimine with IgG monoclonal
20 antibody

The isothiocyanate from Example DD, 4 mg (20 µ
moles) was mixed with 200 µl of 3 mM indium chloride
(60 µ moles). A 20 µl aliquot of the solution was then
25 spiked with radioactive indium-111 chloride and the pH
adjusted to 9 by the addition of 30 µl 1 N NaOH and 10
µl of 0.1 HCL. The indium chelate was mixed with 150
µl of CC-49 whole antibody IgG, 10 mg/ml in 50 mM HEPES
bufffer at pH 9.5. After 18 hours at room temperature
30 the antibody was recovered by preparative HPLC (column
Dupont Zorbax Biosphere GF 250; eluent 0.25 m sodium
acetate, pH 6); and a UV detector at 254 nm and a
radioactivity detector. The recovered antibody was
35 concentrated on an Amicon membrane and exchanged into

PBS buffer at pH 7.4. The recovered antibody had
specific activity of approximately 0.5 μci/100μg.

5   Example 13: In Vivo localization of ^{111}In labeled
    starburst antibody conjugate.

         The usefulness of the labeled starburst
    antibody conjugate prepared in Example 12 was
10 demonstrated by measuring the uptake of the material by
    a human tumor xenograft in an athymic mouse. Female
    anthymic mice were innoculation subcutaneously with the
    human colon carcinoma cell line, LS-174T (approximately
    4×10^6 cells/animal). Approximately two weeks after
15 innoculation, each animal was injected via the tail
    vein. The mice were sacrificed after 17 and 48 hours
    (five animals at each time point), the tumor and
    selected tissues were excised and weighed, and
    radioactivity was measured in a gamma counter. After
20 17 hours 13.5 percent of the injected dose per gram of
    tissue had localized at the tumor. After 48 hours
    21.6% of the injected dose per gram of tissue had
    localized at the tumor.

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1.   A starburst conjugate which comprises at
least one starburst polymer associated with at least
one unit of at least one carried pharmaceutical
material.

2.   The conjugate of Claim 1 wherein the
starburst polymer is a starburst dendrimer.

5

3.   The conjugate of Claim 1 or 2 wherein at
least one of the carried pharmaceutical materials is a
drug, radionuclide, chelant, chelated metal, toxin,
antibody, antibody fragment, antigen, signal generator,
10   signal reflector, or signal absorber.

4.   The conjugate of Claim 2 wherein there are
at least two different carried materials at least one
of which is a target director and at least one of which
15   is a bioactive agent.

5.   The conjugate of Claim 4 wherein the
target director is an entity specific for one or more
target receptors and the bioactive agent is a
20   radionuclide, drug, or toxin.

25

6.   The conjugate of Claim 4 or 5 wherein the
target director is a polyclonal antibody or fragment
thereof.

7.   The conjugate of Claim 4 or 5 wherein the
target director is a monoclonal antibody or fragment
thereof.

8.   The conjugate of Claim 1 wherein the
dendrimer contains discontinuities.

9.   A starburst conjugate of Claim 1 of the
formula:

$$. (P)_x * (M)_y \qquad\qquad (I)$$

wherein each P represents a dendrimer;

x represents an integer of 1 or greater;

each M represents a unit of a carried pharmaceutical
material, said carried pharmaceutical material can be
the same carried pharmaceutical material or a different
carried pharmaceutical material;

y represents an integer of 1 or greater; and

* indicates that the carried pharmaceutical material is
associated with the dendrimer.

10.   The conjugate of Claim 9 wherein M is a
drug, pesticide, radionuclide, chelator, chelated
metal, toxin, antibody, antibody fragment, antigen,
signal generator, signal reflector, or signal absorber.

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11.   The conjugate of Claim 9 wherein x=1 and
y=2 or more.

5   12.   The starburst conjugate of Claim 9 wherein
the molar ratio of any ionic M to P is 0.1-1,000:1.

            13.   The starburst conjugate of Claim 9 wherein
10 the weight ratio of any drug or toxin M to P is 0.1-
5:1.

            14.   A process for preparing

15
                    $(P)_x$ * $(M)_y$ (I)

wherein each P·represents a dendrimer; x represents an
integer of 1 or greater; each M represents a unit of a
20 carried pharmaceutical material, said carried
pharmaceutical material can be the same carried
pharmaceutical material or a different carried
pharmaceutical material; y represents an integer of 1
or greater; and * indicates that the carried
25 pharmaceutical material is associated with the
dendrimer, which comprises reacting P with M, usually
in a suitable solvent, at a temperature which
facilitates the association of the carried material (M)
30 with the starburst dendrimer (P).

            15.   The process of Claim 14 wherein the
temperature is from room temperature to reflux.

35

16. The process of Claim 14 wherein the
suitable solvent is water, methanol, ethanol,
chloroform, acetonitrile, toluene, dimethylsulfoxide or
dimethylformamide.

17. A starburst conjugate of any one of Claims
1 to 11 which also has at least one pharmaceutically
acceptable diluent or carrier present.

18. The starburst conjugate composition of
Claim 17 which also has other active ingredients
present.

19. A starburst conjugate of any one of Claims
1 to 11, 17 and 18 for use as a diagnostic agent.

20. A starburst conjugate of any one of Claims
1 to 11, 17 and 18 for use as a pharmaceutical carrier.

21. A method for the delivery of at least one
carried pharmaceutical material which comprises
administering of at least one starburst conjugate of
any one of Claims 1 to 11, 17 and 18 containing said
material at or near a targeted locus.

22. A method for scavenging therapeutic or
diagnostic compounds which comprises administering of a
bifunctional starburst conjugate containing a target
director of Claim 4 to 7 which localizes the conjugate
to a target locus and a scavenging moiety which can

bind a secondarily administered therapeutic or
diagnostic compound.

23.   The method of Claim 22 wherein the
scavenging moiety is a chelant, antigen, or antibody.

24.   A process for preparing a starburst
conjugate of the formula

$$[(T)_e - (C')_f]_g * (P)_x * [(C'')_h - (M)_y]_k \qquad (III)$$

wherein

each C' represents the same or different connecting
group;

each C'' represents the same or different connecting
group;

g, and k each individually represent an integer of 1 or
greater;

f and h each individually represent an integer of 0 or
greater;

- indicates a covalent bond in instances where a
connecting group is present;

each P represents a dendrimer;

x represents an integer of 1 or greater;

T represents a target director;

each M represents a unit (for exampe, a molecule, atom,
ion, and/or other basic unit) of a carried

pharmaceutical material, said carried pharmaceutical
material can be the same carried pharmaceutical
material or a different carried material, preferably
the carried pharmaceutical material is a bioactive

5 agent;

y represents an integer of 1 or greater; and

* indicates that the carried pharmaceutical material is
associated with the dendrimer;

10

which comprises the reaction of P, having reactive
moieties, with a connecting group such as an aniline
moiety, which may have the NH_2 group protected.

15   24. The process of Claim 23 wherein the
protecting group has the formula

wherein R is $-C(CH_3)_3$,   ,

20

25   ,   or   .

30

35
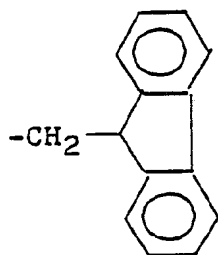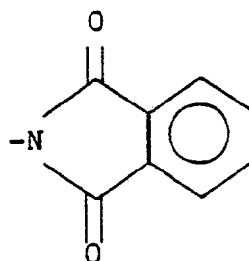
-98-

    25.   The process of Claim 23 wherein the P also
has a connecting group (handle) attached of the formula

5

$$(O_2N)_n \quad \text{—} \quad \bigcirc \quad X$$
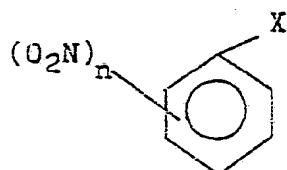
10

where n is 1 or 2, and X is F, Cl, Br, I, SO_2Cl, and
when n is 1, the NO_2 group is in the para position.

    26.   The process of Claim 25 wherein the
15 connecting group is 4-fluoronitrobenzene.

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AMENDED CLAIMS
[received by the International Bureau on 8 January 1988 (08.01.88)
original claims 1 - 26 replaced by new claims 1 - 36 (8 pages)]

1.   A starburst conjugate which comprises at
least one starburst polymer associated with at least
one unit of at least one carried pharmaceutical
material.

2.   The conjugate of Claim 1 wherein the
starburst polymer is a starburst dendrimer.

5

3.   The conjugate of Claim 1 or 2 wherein at
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drug, radionuclide, chelator, chelated metal, toxin,
antibody, antibody fragment, antigen, signal generator,
10   signal reflector, or signal absorber.

4.   The conjugate of Claim 2 wherein there are
at least two different carried materials at least one
of which is a target director and at least one of which
15   is a bioactive agent.

5.   The conjugate of Claim 4 wherein the
target director is an entity specific for one or more
target receptors and the bioactive agent is a
20   radionuclide, drug, or toxin.

25

bind a secondarily administered therapeutic or
diagnostic compound.

23.   The method of Claim 22 wherein the
scavenging moiety is a chelant, antigen, or antibody.

24.   A process for preparing a starburst
conjugate of the formula

$$[(T)_e - (C')_f]_g * (P)_x * [(C'')_h - (M)_y]_k \quad (III)$$

wherein

each C' represents the same or different connecting
group;

each C'' represents the same or different connecting
group;

g, and k each individually represent an integer of 1 or
greater;

f and h each individually represent an integer of 0 or
greater;

- indicates a covalent bond in instances where a
connecting group is present;

each P represents a dendrimer;

x represents an integer of 1 or greater;

T represents a target director;

each M represents a unit (for example, a molecule,
atom, ion, and/or other basic unit) of a carried

pharmaceutical material, said carried pharmaceutical material can be the same carried pharmaceutical material or a different carried material, preferably the carried pharmaceutical material is a bioactive

5   agent;

y represents an integer of 1 or greater; and

* indicates that the carried pharmaceutical material is

10   associated with the dendrimer;

which comprises the reaction of P, having reactive moieties, with a connecting group such as an aniline moiety, which may have the NH_2 group protected.

15   25.   A process for preparing a starburst conjugate as defined in Claim 1 which comprises the reaction of P, having reactive moieties, with a connecting group such as an aniline moiety, which may

20   have the NH_2 group protected.

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26. The process of Claim 25 wherein the
protecting group has the formula

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wherein R is -C(CH$_3$)$_3$, -CH$_2$,

10

  -CH$_2$,   or   .

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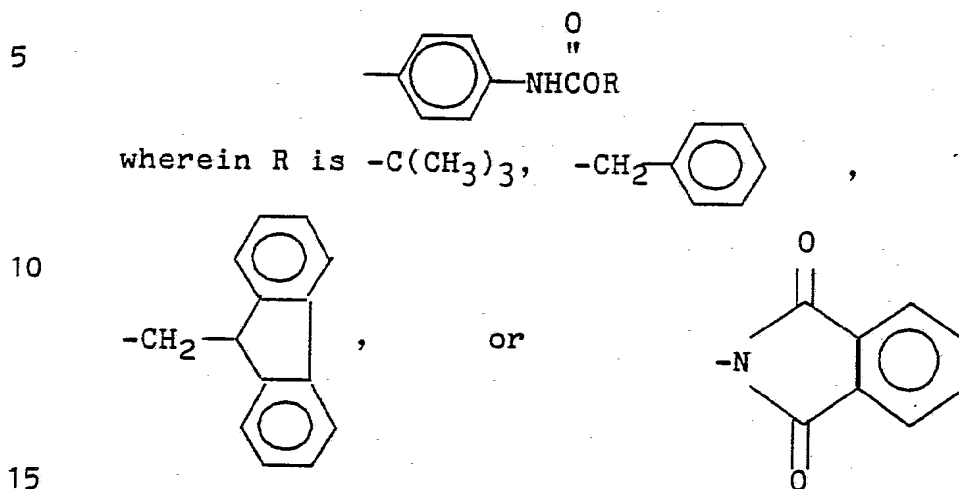
27. The process of Claim 25 wherein the P also
has a connecting group (handle) attached of the formula

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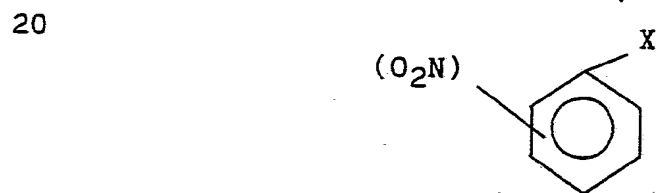
where n is 1 or 2, and X is F, Cl, Br, I, SO$_2$Cl, and
when n is 1, the NO$_2$ group is in the para position.

30   28. The process of Claim 27 wherein the
connecting group is 4-fluoronitrobenzene.

29. The conjugate of Claim 2 wherein the
starburst dendrimer is of the formula

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$$\left(\text{Core} \left\{ \left(\text{Repeat Unit} \right)_{\frac{N_r^{G-1}}{N_r-1}} \quad \frac{\text{Terminal}}{\text{Moiety}} \quad N_r^G \right\} \right)_{N_c}$$
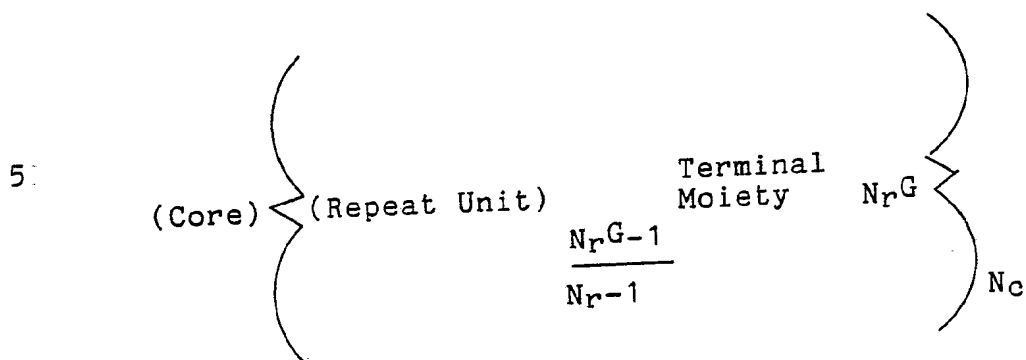
10   wherein: the core is

     # of terminal groups per dendritic branch =

15

$$\frac{N_r^G}{2} \; ;$$

  G is the number of generations; N_r is the repeating
  unit multiplicity which is at least 2; N_c is the
20 valency of the core compound; the terminal moiety is
  determined by the following:

    # of terminal moieties per dendrimer =

25

$$\frac{N_c N_r^G}{2}$$

  wherein N_r, G and N_c are as defined above; and the
30 Repeat Unit has a valency or functionality of $N_r + 1$
  wherein N_r is as defined above.

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30.   The conjugate of Claim 2 wherein the starburst dendrimer is of the formula

$$\left(\begin{array}{c} \text{I} \end{array} (Z^c)_{N_c} \right) \left\{ \left(X^iY^i(Z^i)_{N^i} \right)^{i-1}_{\substack{N_c\pi N^n \\ n\,is\,1}} \right\} \left(X^tY^t(Z^t)_{N^t} \right)^{t-1}_{\substack{N_c\pi N^n \\ n\,is\,1}}$$
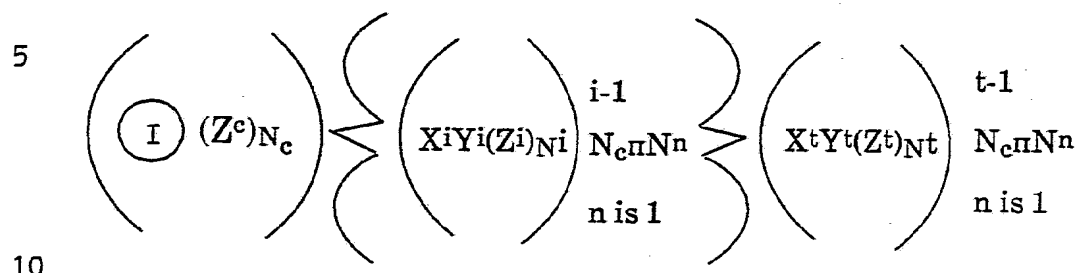
wherein i is 1 to t−1; the core compound is represented by the formula

$$\text{I} \; (Z^c)_{N_c}$$

where

$$\text{I}$$

represents the core, Z^c represents the functional groups bonded to

$$\text{I}$$

and N_c represents the core valency; the repeat unit is represented by the formula $X^iY^i(Z^i)_{N^i}$ wherein "i" is defined as above; the final or terminal units are represented by $X^tY^t(Z^t)_{N^t}$ wherein t represents terminal generation and X^t, Y^t, Z^t and N^t may be the same as or different from X^i, Y^i, Z^i and N^i except that there is no succeeding generation connected to the Z^t groups and N^t may be less than two; the n function is the product of all the values between its defined limits, such as

$$\text{Nn} = \prod_{n=1}^{i-1} (N^1)(N^2)(N^3)...(N^{i-2})(N^{i-1})$$

which is the number of repeat units, $X^i Y^i (Z^i)_{N^i}$, comprising the ith generation of one dendritic branch and when i is 1, then $\prod_{n=1}^{i-1} n^0 = 1$.

31. A process for preparing a starburst conjugate as defined in Claim 1 which comprises the reaction of P, having reactive moieties, with an aniline moiety, which may have the NH$_2$ group protected by an N-phthalimide of the formula
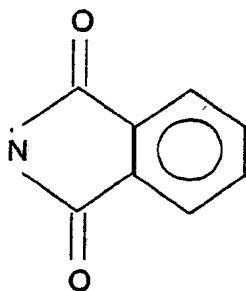
32. A process for preparing a starburst conjugate as defined in Claim 1 which comprises the reaction of P, having reactive moieties, which may have the NH$_2$ group protected by any protecting group used for amines which is inert under the conditions used for starburst synthesis.

33. A process for preparing a starburst polyethyleneimine which comprises reacting a starburst polyethyleneiminemethane sulfonamide with hydrochloric acid.

34. A process for purifying a starburst
dendrimer having a solvent present which comprises
removing the solvent by ultrafiltration using a
membrane.

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35. The process of Claim 34 wherein the
solvent is ethylenediamine.

36. A process for preparing a starburst
10 conjugate of Claim 1 wherein the starburst polymer is a
starburst dendrimer and the carried material is a
lanthanide which comprises chelation of the lanthanide
with a starburst polyethyleneimine acetate.

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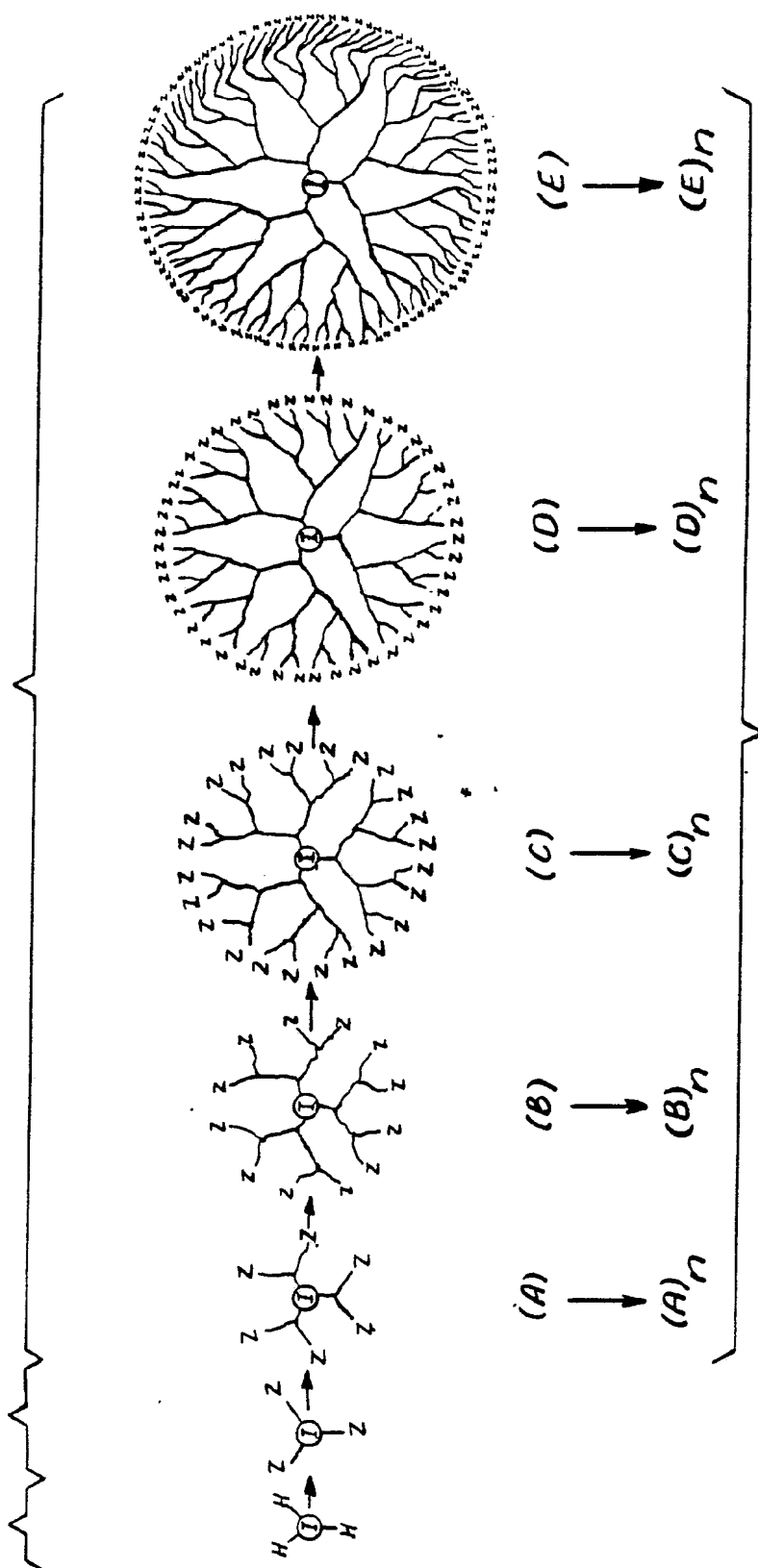
Fig. 1
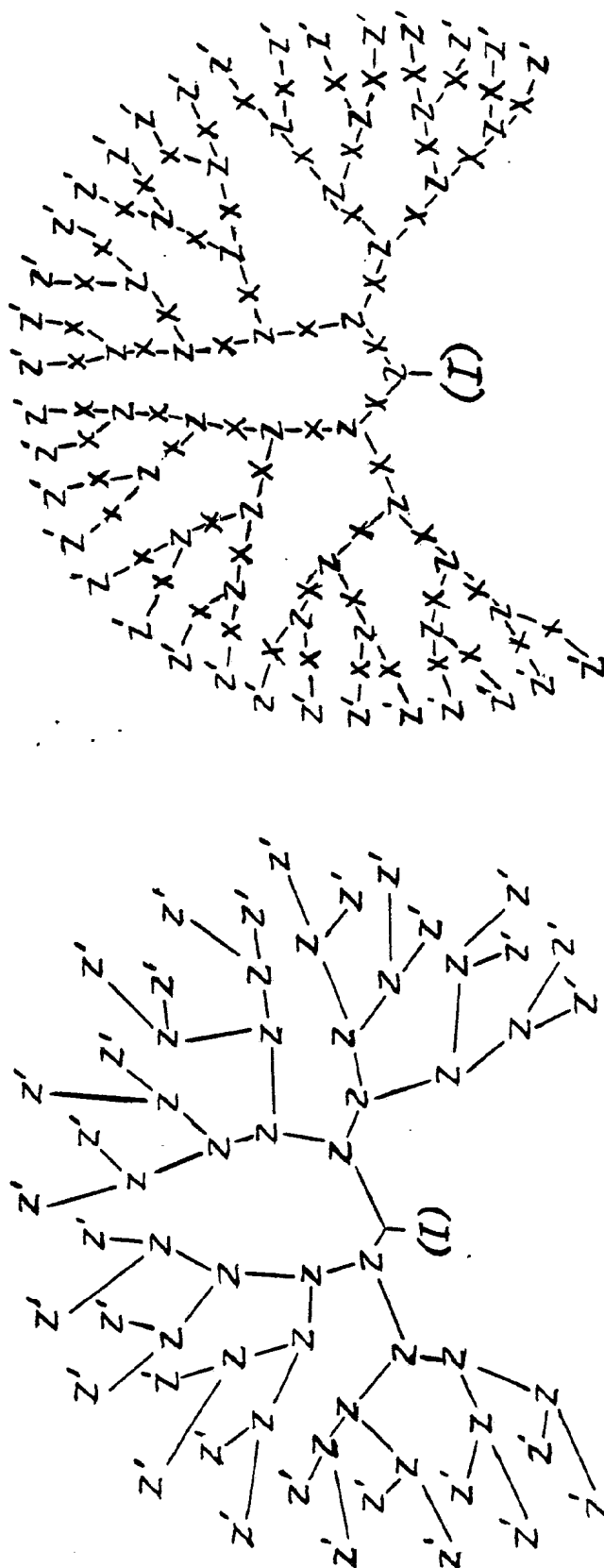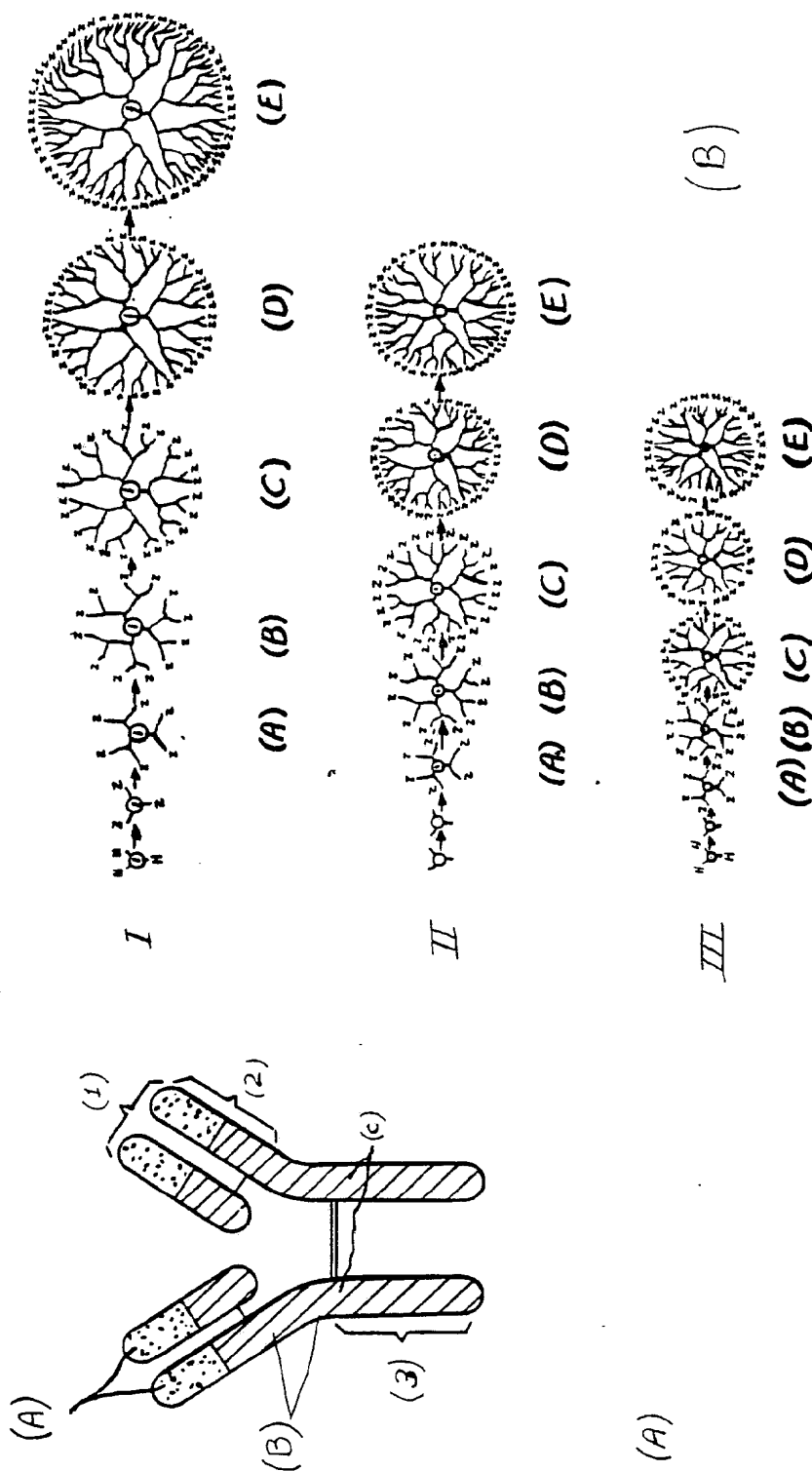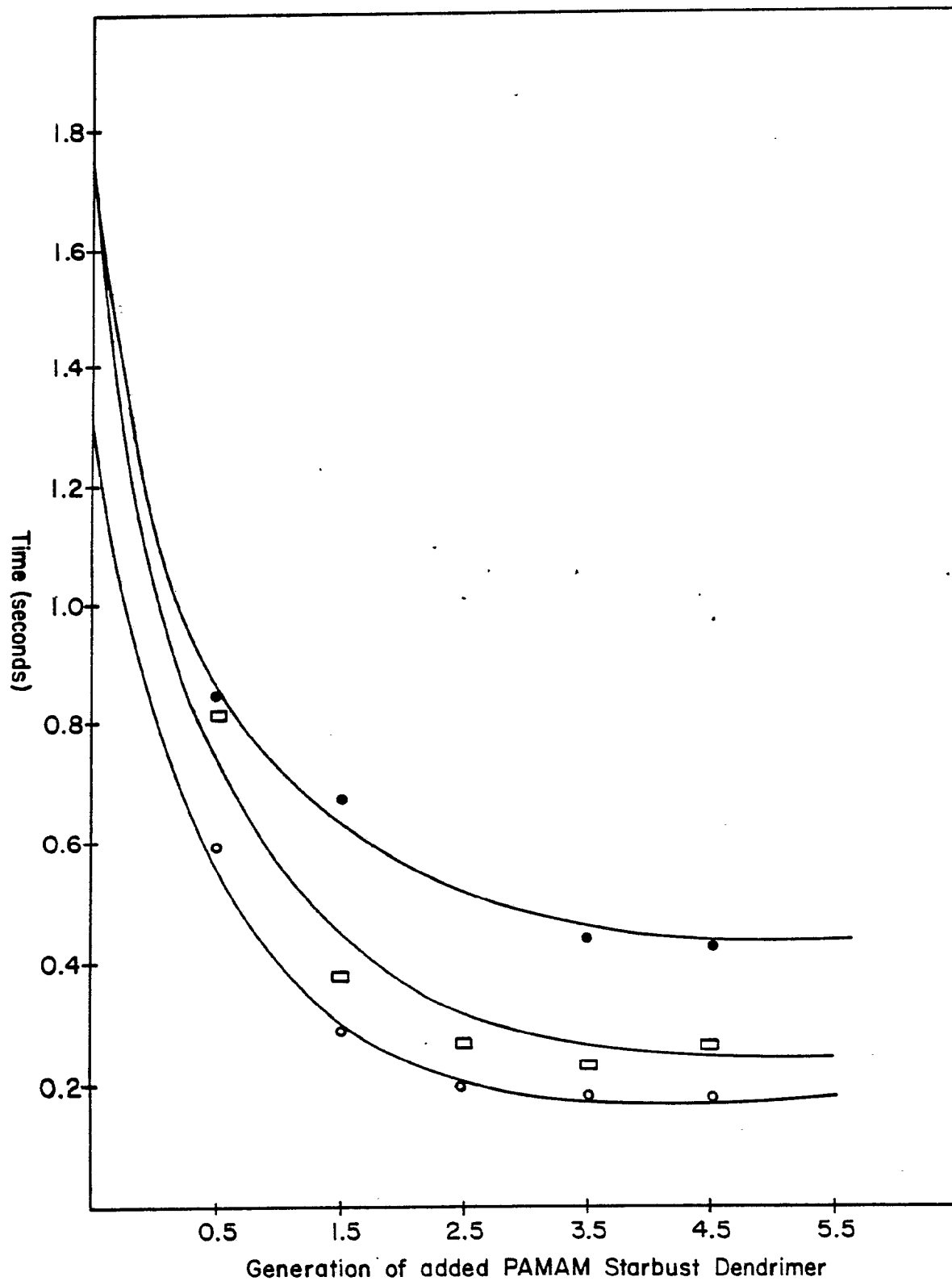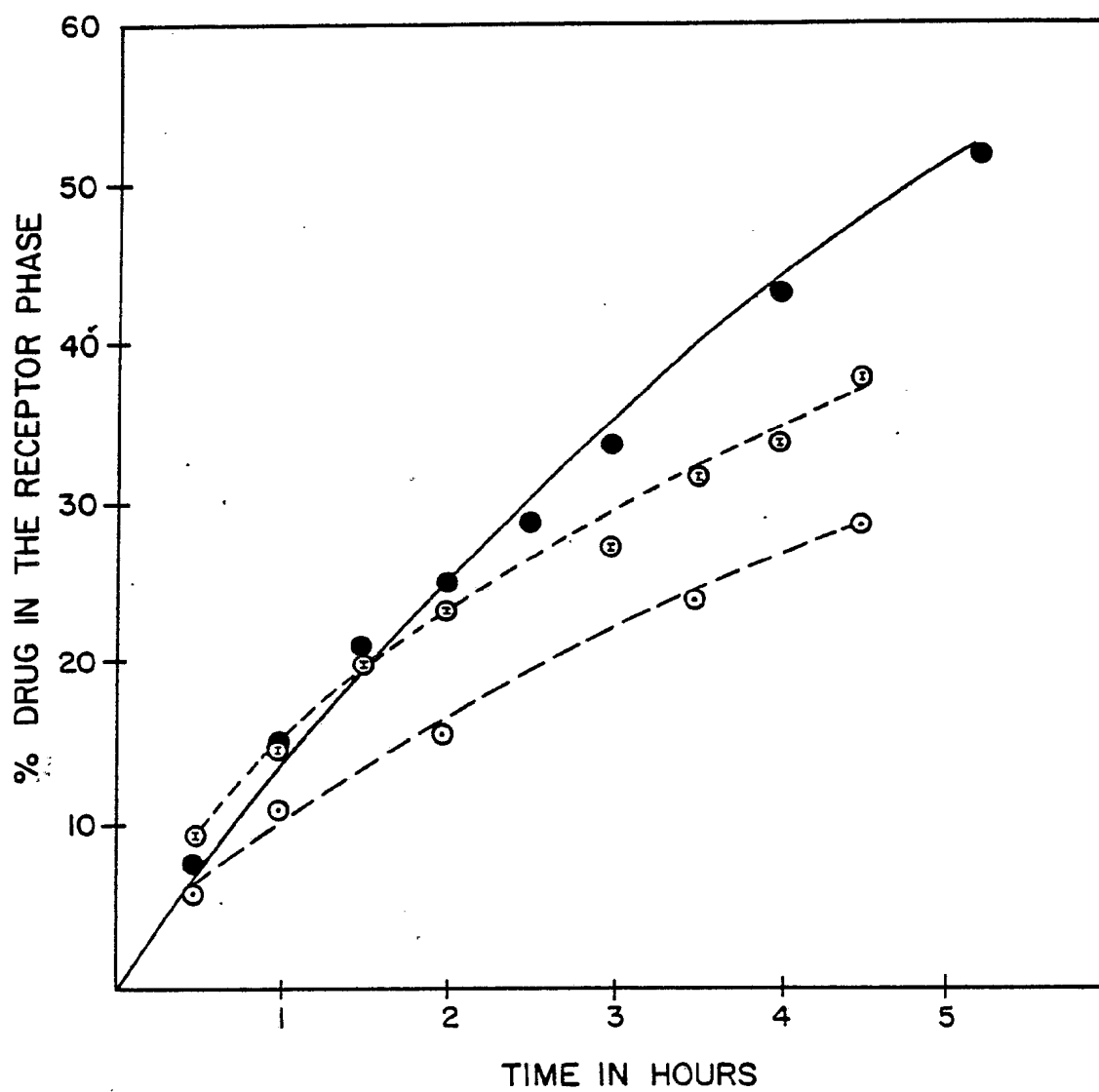
Fig. 2

FIG. 3

FIG. 4

FIG. 5

FIG. 6
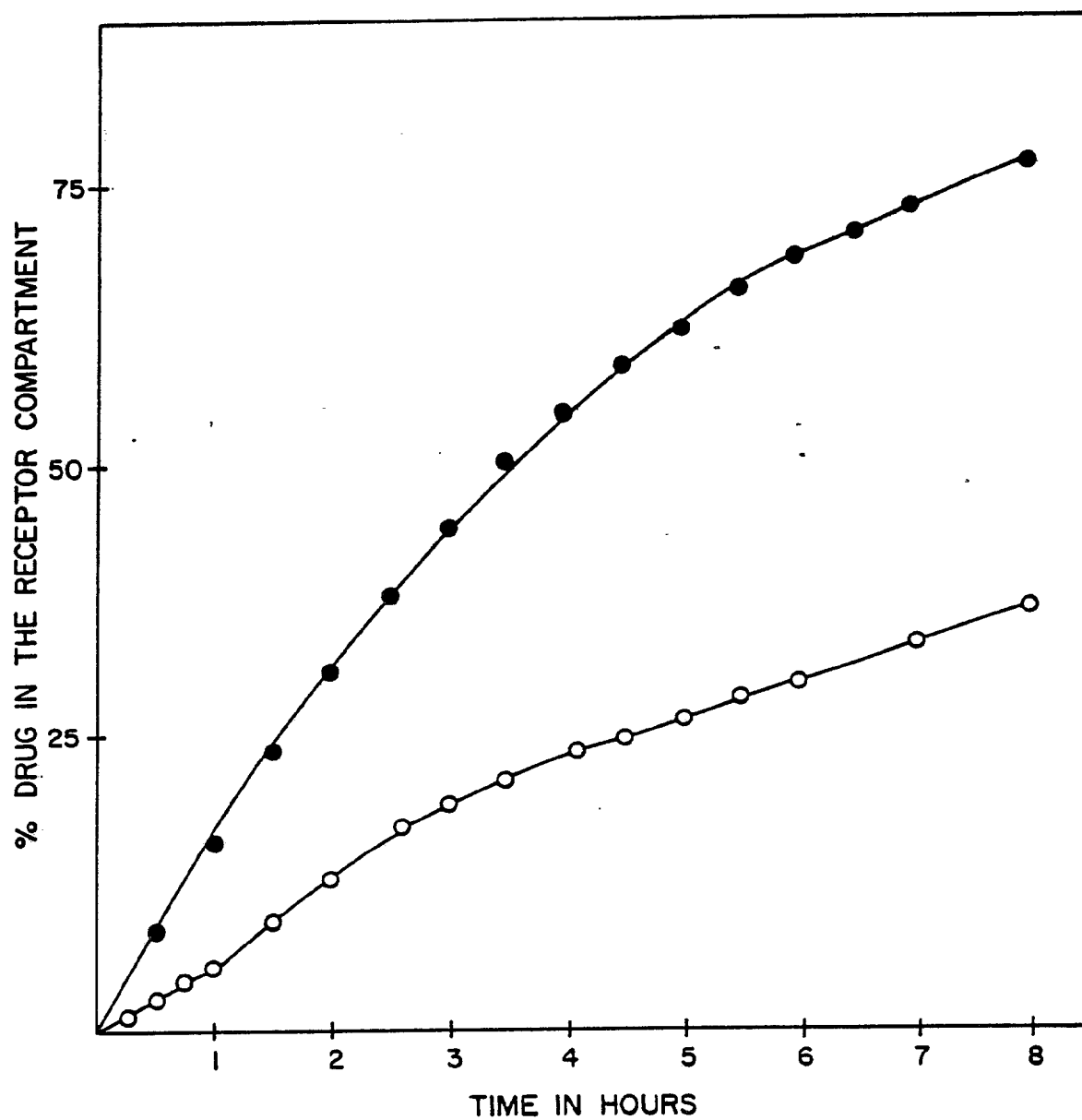
FIG.7
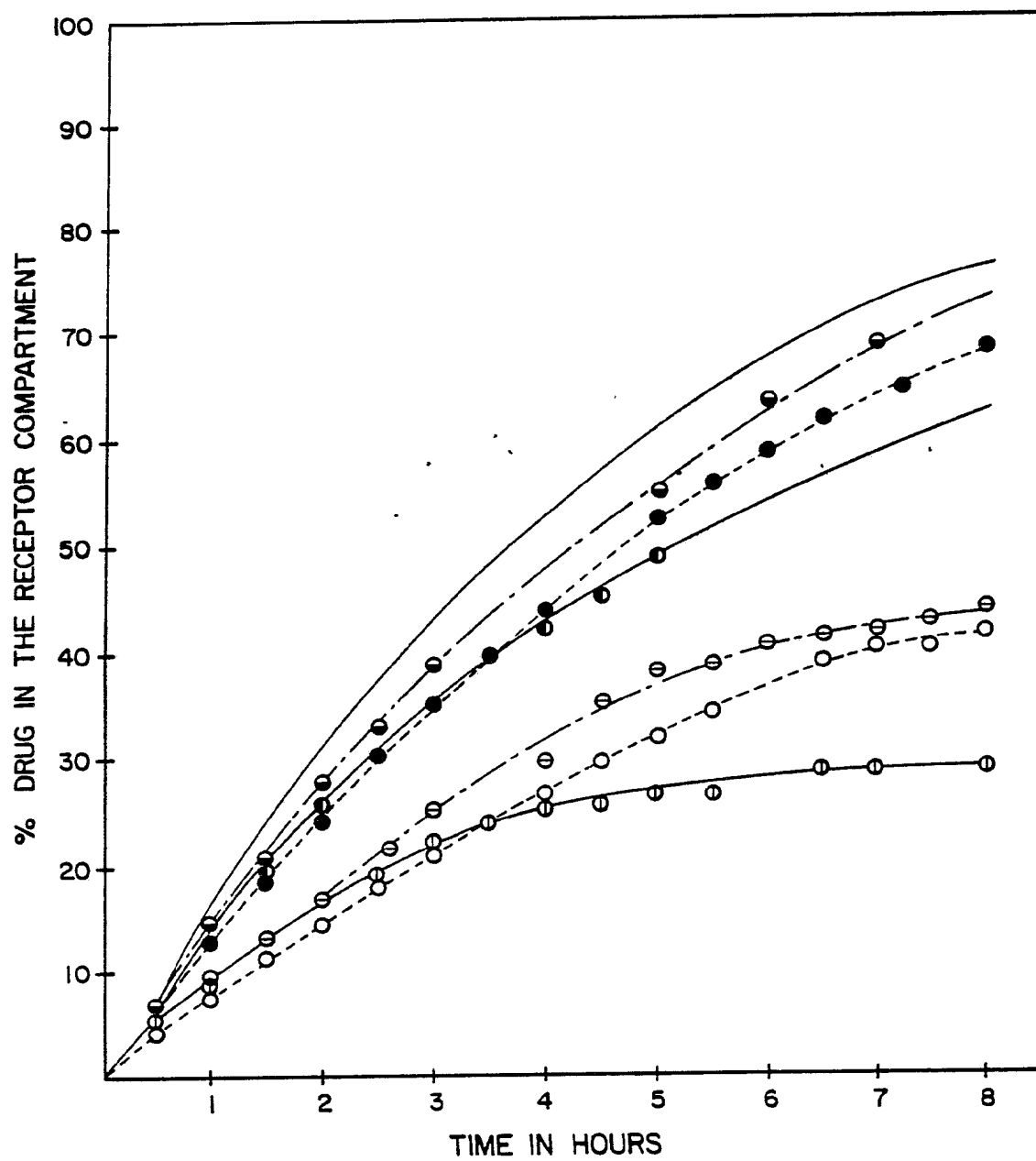
FIG. 8
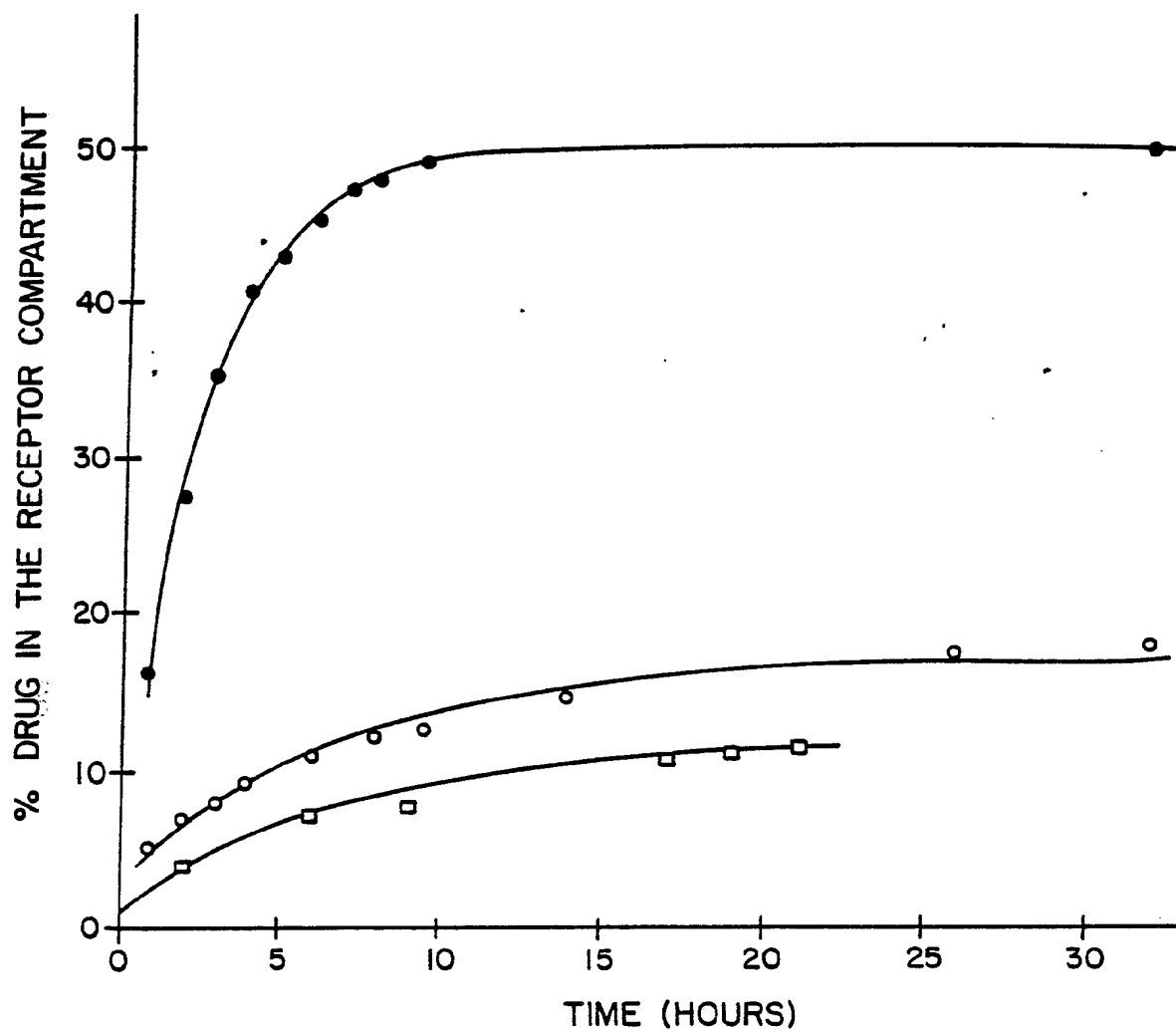
FIG. 9
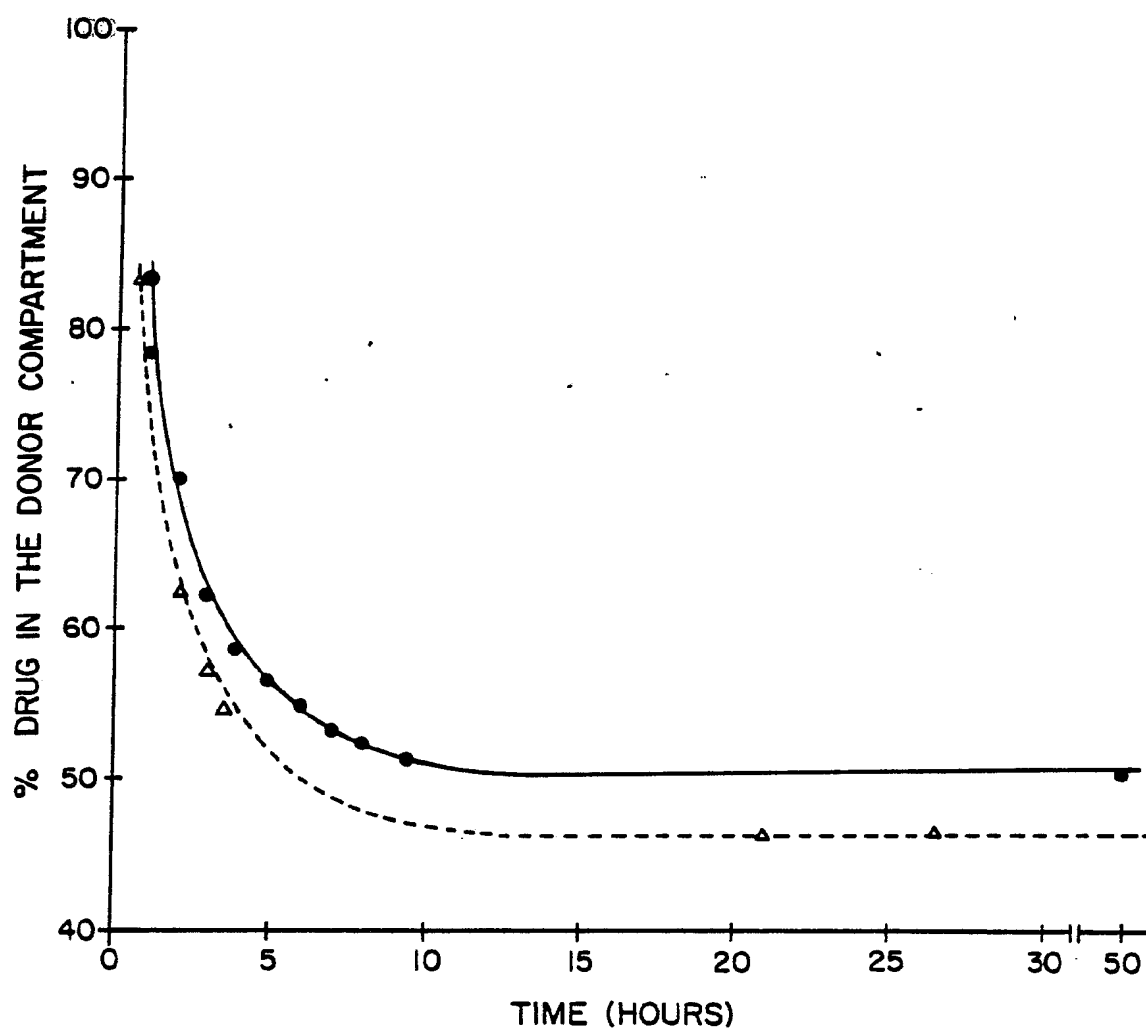
FIG. 10
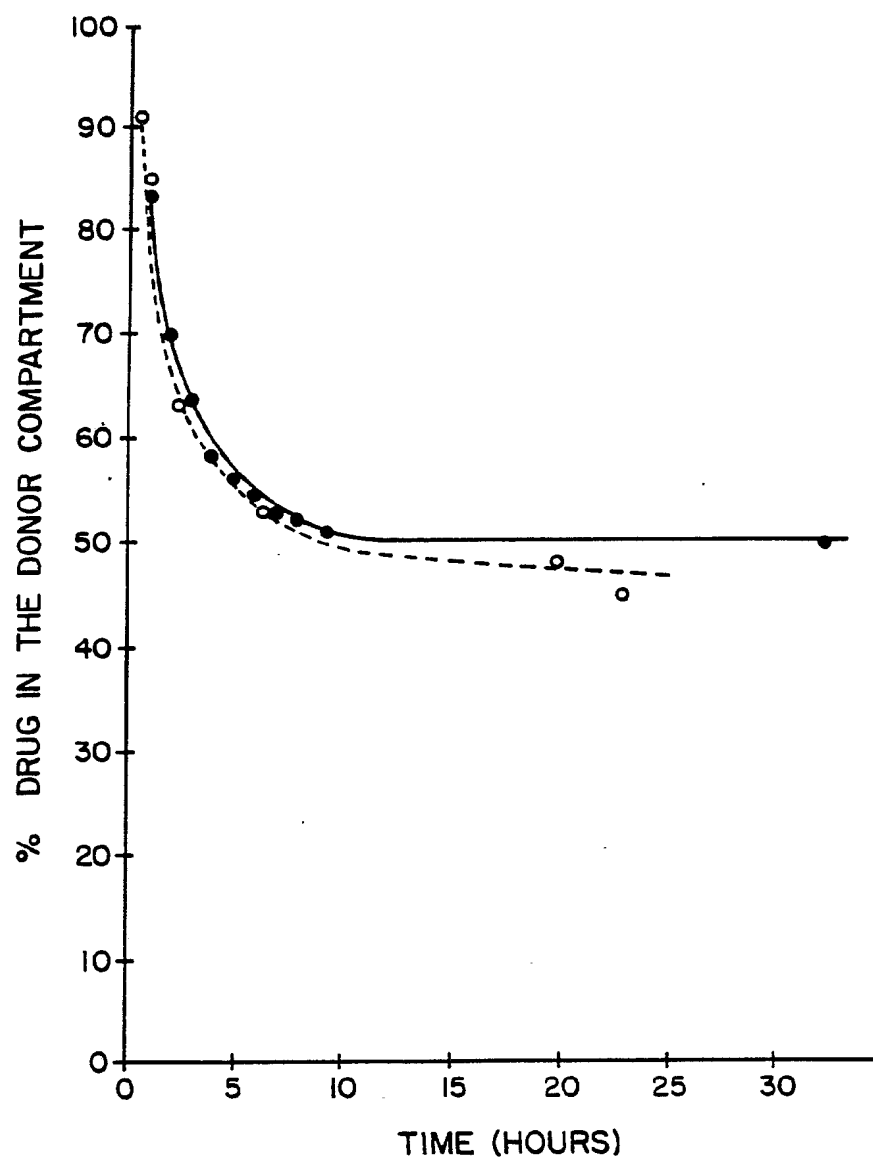
INTERNATIONAL SEARCH REPORT

International Application No PCT/US 87/02074

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

INT.CL. [4] A61K 49/02
US. CL. 424/1.1

II. FIELDS SEARCHED

Minimum Documentation Searched [4]	
Classification System	Classification Symbols
U.S.	424/1.1,9 525/410,416,418,451 528/310,332,350,363,397

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched [5]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category [*]	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
A	US, A, 4,558,120 PUBLISHED 10 DECEMBER 1985 TOMALIA ET AL	1-27
P,A	US, A, 4,606,907 PUBLISHED 19 AUGUST 1986 SIMON ET AL	1-27
T	US, A, 4,694,064 PUBLISHED 15 SEPTEMBER 1987 TOMALIA ET AL	1-27

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
29 OCTOBER 1987	2 4 NOV 1987
International Searching Authority [1]	Signature of Authorized Officer [20]
ISA/US	John Maples

Form PCT/ISA/210 (second sheet) (May 1986)

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V.☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [10]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1.☐ Claim numbers .... , because they relate to subject matter [12] not required to be searched by this Authority, namely:

2.☐ Claim numbers........ , because they relate to parts of the international application that do not comply with the prescribed require-
ments to such an extent that no meaningful international search can be carried out [13], specifically:

VI.☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [11]

This International Searching Authority found multiple inventions in this international application as follows:

    I.   Claims 1-23 drawn to a starburst conjugate and process
        of preparing classified in class 424 subclass 1.1.
    II.   Claims 24-27 drawn to a process of preparing a starburst
        conjugate classified in class 528 subclass 363.

1.☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims
of the international application.

2.☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only
those claims of the international application for which fees were paid, specifically claims:

3.☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to
the invention first mentioned in the claims; it is covered by claim numbers:

4.☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not
invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (supplemental sheet (2) (May 1986)